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REVIEW ARTICLE

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The *Yersinia* high-pathogenicity island

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Summary A pathogenicity island present only in highly pathogenic strains of *Yersinia* (*Y. enterocolitica* 1B, *Y. pseudotuberculosis* I and *Y. pestis*) has been identified on the chromosome of *Yersinia* spp. and has been designated High-Pathogenicity Island (HPI). The *Yersinia* HPI carries a cluster of genes involved in the biosynthesis, transport and regulation of the siderophore yersiniabactin. The major function of this island is thus to acquire iron molecules essential for in vivo bacterial growth and dissemination. The presence of an integrase gene and *att* sites homologous to those of phage P4, together with a G + C content much higher than the chromosomal background, suggests that the HPI is of foreign origin and has been acquired by chromosomal integration of a phage. The HPI can excise from the chromosome of *Y. pseudotuberculosis* and is found inserted into any of the three copies of the *asn* tRNA loci present in this species. A unique characteristic of the HPI is its wide distribution in various enterobacteria. Although first identified in *Yersinia* spp., it has subsequently been detected in other genera such as *E. coli*, *Klebsiella* and *Citrobacter*.

Key words *Yersinia* · Pathogenicity island · Siderophore · Yersiniabactin · Microbial pathogenesis

Introduction

The genus *Yersinia* belongs to the family *Enterobacteriaceae* and is composed of 11 species. Only three are pathogenic for humans and animals: *Y. pestis* is the causal agent of bubonic and pneumonic plague, whereas *Y. enterocolitica* and *Y. pseudotuberculosis* are enteropathogenic bacteria transmitted by the oral route and responsible for intestinal symptoms. All pathogenic strains of *Yersinia* carry a conserved 70 kb plasmid, pYV (for plasmid associated with *Yersinia* virulence). This plasmid is essential for virulence and its presence therefore differentiates pathogenic from non-pathogenic *Yersinia* (Table 1).

Pathogenic *Yersinia* can be further subdivided into low-pathogenicity strains, i.e. strains that induce a mild intestinal infection in humans and are non-lethal for mice at low doses, and high-pathogenicity strains which cause severe systemic infections in humans and kill mice at low doses. This difference in the level of virulence correlates with the presence of a large chromosomal fragment (Table 1) which has the characteristics of a pathogenicity island. As the presence of this region correlates with the expression of a high-pathogenicity phenotype in *Yersinia*, it has been called “High-Pathogenicity Island” or HPI [6].

What is a pathogenicity island ?

The term ‘pathogenicity island’ was coined by Hacker et al. to describe two large unstable regions of the chromosome of uropathogenic *E. coli* [25]. This term usually refers to a large (35 kb) chromosomal segment that carries genes involved in pathogenicity. Characteristically, pathogenicity islands are bordered on one side by a tRNA gene and less frequently, may be flanked by insertion sequences. These islands are often unstable and their spontaneous deletion occurs at frequencies of 10^{-4} to 10^{-5}. Their G + C content is usually different from the rest of the host chromosome, suggesting that they originate from horizontal transfer between different bacterial genera [26]. The number of Gram-negative bacterial species shown to harbor pathogenicity islands has grown steadily and now includes uropathogenic and enteropathogenic *E. coli*, *Helicobacter pylori*, *Salmonella typhimurium*, *Dichelobacter nodosus* and *Vibrio cholerae* [26].

Table 1 Subdivision of *Yersinia* strains into three pathogenicity groups based on the presence of the pYV virulence plasmid and the high-pathogenicity island

Genus *Yersinia*		
Pathogenic (= pYV+)		Non-pathogenic (= pYV–)
High-pathogenicity (= HPI+)	Low-pathogenicity (= HPI–)	
Y. pestis	*Y. pseudotuberculosis* II, IV, V	*Y. enterocolitica* 1A
Y. pseudotuberculosis (III)	*Y. enterocolitica* 2–5	*Y. intermedia*
Y. enterocolitica 1B		*Y. kristensenii*
		Y. frederiksenii
		Y. mollaretii
		Y. bercovieri
		Y. aldovae
		Y. rohdei
		Y. ruckeri

Characteristics of the High-Pathogenicity Island of *Yersinia*

Overall structure of the HPI The *Yersinia* HPI has characteristics typical of a pathogenicity island in that: (i) it is a large chromosomal DNA fragment, (ii) it carries virulence genes, namely the yersiniabactin system, involved in siderophore-mediated iron-acquisition [21, 23, 37] and essential for the expression of the high-virulence phenotype, (iii) it incorporates several repeated sequences (IS*1328*, IS*1400* and RS3), (iv) it is bordered on one side by a tRNA gene (*asn* tRNA), and (v) its G + C content is different from that of the rest of the chromosome. The size of the HPI of highly pathogenic *Yersinia* is between 35 and 45 kb depending on species. The ²32 kb right-hand part of the HPI is highly conserved in the three pathogenic species *Y. pestis*, *Y. pseudotuberculosis* and *Y. enterocolitica*, whereas the left-hand part of the island is less well conserved (Fig. 1). As most of the genes located on the HPI are involved in siderophore-mediated iron acquisition, the HPI may be regarded as an iron-capture island. The few remaining genes located on the HPI are mobility genes (insertion sequences or bacteriophage genes).

The yersiniabactin-mediated iron-capture machinery Highly pathogenic *Yersinia* secrete a siderophore called yersiniabactin [29] which chelates iron molecules bound to eukaryotic proteins and transports them back into the bacterium. This 482 Da molecule belongs to a small subgroup of phenolate siderophores and has a high affinity for ferric ions with a K_D of 4×10^{-36} [21]. The structure of yersiniabactin (also called yersiniophore) has now been determined [11, 16, 24]. It contains a benzene and a thiazolidine ring, and two thiazoline rings. The structure of yersiniabactin is very similar to those of pyochelin and anguibactin, produced by *Pseudomonas aeruginosa* and *Vibrio anguillarum*, respectively.

The locus involved in yersiniabactin-mediated iron acquisition is located on the HPI and is composed of 11 genes organized into four operons (Fig. 1) that have been partially sequenced in *Y. enterocolitica* [23, 37, 45] and completely sequenced in *Y. pestis* [1, 17, 18, 21]. The exact function of all these genes has not yet been entirely elucidated but they can be roughly divided into three functional groups: yersiniabactin biosynthesis, transport into the bacterial cell (outer membrane receptor and transporters), and regulation. The correspondence between gene designations in *Y. pestis* and *Y. enterocolitica* 1B is given in Table 2, along with the hypothetical or confirmed function of each gene.

YbtE and the first part of HMWP2 (high molecular weight protein 2) are responsible for initiating assembly of the yersiniabactin molecule [22]. Siderophore synthesis then proceeds in a modular fashion, using a mixed polyketide synthase/nonribosomal peptide synthetase complex formed between HMWP1 and HMWP2 [21]. YbtS is also essential for yersiniabactin synthesis, probably for converting chorismate to salicylate. YbtT possesses a thioesterase-like domain, suggesting that it plays a role in yersiniabactin biosynthesis. The functions of YbtX and YbtU have not yet been determined.

Three genes are involved in yersiniabactin-mediated internalization of iron: *psn/fyuA*, *ybtP* and *ybtQ*. Psn/FyuA is the outer membrane receptor for yersiniabactin and the bacteriocin, pesticin [18, 19, 30, 45]. It contains a region commonly present in Ton-B-dependent proteins [18, 30]. YbtP and YbtQ are inner-membrane permeases required for the translocation of iron into the bacterial cytosol [21]. Whether yersiniabactin is concomitantly internalized during

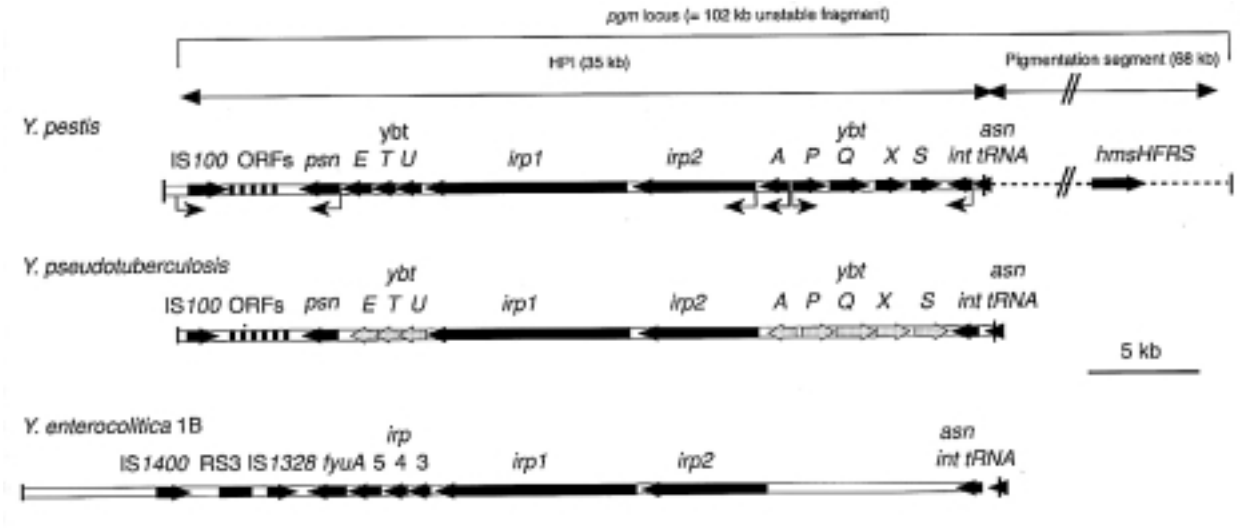

Fig. 1 Genetic organization of the HPI of the three highly pathogenic *Yersinia* species. The HPI are represented by a thick dotted line. Dotted thin line corresponds to adjacent chromosomal DNA. Black arrows indicate genes that have been completely (*Y. enterocolitica* and *Y. pestis*) or partially (*Y. pseudotuberculosis*) sequenced. Gray arrows on the genetic map of *Y. pseudotuberculosis* represent genes that have not been sequenced but that are assumed to be present at the same position as in *Y. pestis* based on hybridization experiments. The arrows below the genetic map of *Y. pestis* indicate the various operons. ORFs correspond to a cluster of short open reading frames that may correspond to cryptic genes of phage origin

this process is not clear.

YbtA belongs to the AraC family of transcriptional regulators [17]. It activates expression from the *psn*, *irp2* and *ybtP* promoters but represses expression from its own promoter. All four promoter regions (*psn*, *irp2*, *ybtA* and *ybtP*) of the yersiniabactin region possess a Fur-binding site and are negatively regulated by this repressor in the presence of iron [21, 23, 45]. The Fur repressor has been identified and characterized in *Y. pestis* [49, 50]. There is some evidence that yersiniabactin itself may upregulate its own expression and that of *psn/fyuA* [1, 17, 18, 37].

Table 2 Genes composing the yersiniabactin locus and their putative functions in *Yersinia* (derived from [21])

Gene designation in *Yersinia pestis*	Gene designation in *Yersinia enterocolitica*	Function
ybtS	NS	Silicylate synthesis
ybtX	NS	UN
ybtQ	NS	Inner membrane permease
ybtP	NS	Inner membrane permease
ybtA	NS	Activator
irp2[a]	*irp2*	Peptide synthethase
irp1[b]	*irp1*	Peptide/polyketide synthethase
ybtU	*irp3*	UN
ybtT	*irp4*	Thioesterase?
ybtE	*irp5*	Salicyl-AMP ligase
psn	*fyuA*	Outer membrane receptor

NS: Not yet sequenced, UN: Unknown.
[a] *irp2* encodes the high molecular weight protein 2 (HMWP2).
[b] *irp1* encodes HMWP1.

The yersiniabactin machinery is highly conserved in *Y. enterocolitica* 1B and *Y. pestis*, with more than 98% identity between the genes sequenced in the two species [21]. Although the yersiniabactin locus has not yet been sequenced in *Y. pseudotuberculosis*, the perfect conservation of the HPI restriction map with respect to that of *Y. pestis*, the high nucleotide identity observed upon sequencing of short portions of genes [44] and hybridization experiments with *Y. pestis* probes suggest that the yersiniabactin machinery is also highly conserved in *Y. pseudotuberculosis* I [3].

Other genes located on the HPI Most of the HPI encodes the yersiniabactin machinery. The few other genes identified so far on the island are considered to be mobility genes.

One of these sequences (*int*), located in the right-hand part of the HPI (Fig. 1), is homologous to the integrase gene of bacteriophage P4 [3]. This locus is present and conserved in the three pathogenic species of *Yersinia* [3, 27, Buchrieser et al., submitted, Bach et al., in preparation]. The presence of this integrase gene at one extremity and of cryptic genes homologous to bacteriophage genes at the other extremity of the HPI of *Y. pestis* [Buchrieser et al., submitted] argues for the acquisition en bloc of the island, by horizontal transfer via a bacteriophage.

The HPI also carries genes encoding one or several insertion elements. In *Y. pestis* and *Y. pseudotuberculosis* I, a single insertion sequence, IS*100* [19, 42, 43], is present on the HPI (Fig. 1), close to its left-hand border [3]. At approximately the same position on the *Y. enterocolitica* 1B HPI, there is no IS*100* copy but there is a cluster of three different sequences that are repeated elsewhere on the chromosome [6]. Two of these correspond to the insertion sequences IS*1328* [44] and IS*1400* [6] and the third (RS3) has not yet been characterized.

The remaining 10 kb portion of the HPI of *Y. enterocolitica* 1B, corresponding to the left-hand part of the island and including the cluster of repeated sequences, is not highly conserved among the various strains of this species [6] and is absent from the *Y. pestis* and *Y. pseudotuberculosis* HPI. This region has not yet been sequenced.

HPI mobility

Three observations indicate that the HPI arrived in *Yersinia* from another bacterium via a bacteriophage: (i) the G + C content of the open reading frames located on the *Yersinia* HPI is close to 60% [37, Buchrieser et al., submitted] and therefore much higher than the 46–50% reported for the rest of the chromosome, (ii) the HPI carries an integrase gene homologous to the phage P4 integrase gene, and (iii) the HPI of the three pathogenic species of *Yersinia* is integrated into an asparagine tRNA (*asn* tRNA) gene [3, 4, 6, 27] and it is known that tRNA loci are preferential sites for phage integration.

Precise excision of the HPI of *Y. pseudotuberculosis* I occurs spontaneously at a frequency of $\geq 10^{-4}$ [3]. In this species, a 17 bp sequence located at the 3′ end of the *asn* tRNA locus and resembling the phage P4 attachment site (*att*) is repeated at the other extremity of the island [3]. In HPI-deleted organisms, the flanking chromosomal segments have recombined upon HPI excision with the loss of one *att* site and restoration of an intact *asn* tRNA locus. These observations suggest that site-specific excision of the *Y. pseudotuberculosis* island mediated by the HPI-encoded P4-like integrase occurs at the *att*-like sites. Little is known about pathogenicity island integration. In *Y. pseudotuberculosis*, it has been shown that the HPI may be inserted into any of the *asn* tRNA loci present on the chromosome of this species, suggesting that the three copies of this gene have similar potentials for HPI integration [3]. Within a single strain (IP32637), individual colonies where found to have HPI insertions into two different *asn* tRNA loci, supporting the hypothesis that this element retains the ability to excise and reintegrate into the chromosome.

In contrast to the situation in *Y. pseudotuberculosis*, the excision of the *Y. pestis* HPI is not precise but occurs as part of a much larger 102 kb chromosomal deletion. This unstable region is called the *pgm* locus [20] because its presence confers a pigmented-like phenotype on colonies grown at 28°C in the presence of hemin or Congo-red dye [33, 51]. This locus encompasses part of the HPI extending from the IS*100* copy to the right-hand border (Fig. 1) and extends further rightward over the ca. 68 kb pigmentation segment [4] that carries the *hms* locus (for hemin storage). In vitro, the *hms* locus is responsible for the pigmented phenotype of *Y. pestis* [36, 38–41] but its physiological function appears to be the blockage of the flea proventriculus [31, 35]. The 102 kb unstable segment is flanked by IS*100* elements, homologous recombination between which probably accounts for its spontaneous excision [19, 20] at a frequency of 10^{-5} [2]. On serial subculture of a single *Y. pestis* colony, the proportion of HPI-deleted mutants rises, with almost 100% deleted colonies after 16 subcultures [14]. Although precise excision of the HPI of *Y. pestis* has never been reported, this island may still have the capacity to excise because it possesses the 17 bp repeats at each extremity and an intact P4-like integrase gene [27, Buchrieser et al., submitted]. The high deletion frequency of the 102 kb *pgm* locus may mask the lower deletion frequency of the HPI alone.

The HPI of *Y. enterocolitica* 1B appears to be the most stable. Spontaneous deletions are not seen in wild-type isolates or following serial subcultures in vitro [14]. Various deletions covering different portions of the HPI have been reported [44] but no precise excision of the island has ever been found. A P4-like integrase gene is present at the same position on the *Y. enterocolitica* 1B HPI as in *Y. pseudotuberculosis* and *Y. pestis*, but this gene is interrupted by a stop codon [Bach et al., in preparation]. Moreover, the 17 bp *att*-like site is present at the right-hand border of the island but is degenerate at the other extremity [Bach et al., in preparation]. Therefore, the absence of a functional integrase and of conserved direct repeats

at each extremity of the island may account for the stability of the HPI of *Y. enterocolitica* 1B.

How does the HPI enhance *Yersinia* pathogenicity?

The role of the HPI in pathogenesis Numerous reports have demonstrated a role of the HPI in *Yersinia* pathogenesis. Investigation of large numbers of *Yersinia* species indicates that the presence of HPI-specific genes or products correlates with the level of pathogenicity of these natural isolates [9, 10, 12, 14, 28–30, 46]. In *Y. pestis*, non-pigmented strains (due to spontaneous deletion of the unstable 102 kb fragment [19]) have low virulence in mice [8, 32, 34, 52]. In *Y. enterocolitica* 1B, abolition of yersiniabactin production by Tn*5* insertions alters the pathogenicity of the mutants [29]. Finally, specific mutations of *irp2* and *fyuA/psn* in various highly pathogenic species of *Yersinia* show that both genes are important for the expression of the high-pathogenicity phenotype (Table 3). Other yersiniabactin operon genes have not yet been mutated to evaluate their role in virulence.

Virulence properties conferred by the HPI Iron acts as a cofactor in a number of enzymatic and metabolic pathways and is an essential element for almost all bacteria. Pathogenic bacteria encounter conditions in which the concentration of free iron in the host is too low to support their growth. In mammals, iron is either sequestered intracellularly or is bound to specific carrier proteins such as lactoferrin and transferrin. In response to infection, the availability of free iron in body fluids is further reduced by shifting iron from transferrin to lactoferrin in the liver [5]. This process is called induced hypoferremia and forms part of the non-specific immune response. For bacterial pathogens, the successful establishment of disease depends on the ability of the invading organism to acquire iron. One strategy for acquiring iron molecules complexed to host proteins is to synthesize and secrete high-affinity iron compounds called siderophores. Siderophore production can therefore be regarded as a virulence factor in many bacteria.

In *Yersinia*, although other unidentified factors may contribute to the high-pathogenicity phenotype, one of the major differences between low and high-pathogenicity strains lies in their ability to capture iron molecules in vivo and thus to disseminate and cause systemic infections. As early as 1956, Jackson and Burrows observed that attenuated non-pigmented strains of *Y. pestis* (now known to be deficient in yersiniabactin secretion due to deletion of the 102 kb segment) could regain virulence if iron was injected into animals prior to bacterial challenge [34]. A more recent study demonstrated that high-pathogenicity *Y. enterocolitica* strains of serotype O:8 (biotype 1B) are inherently lethal for laboratory animals at low doses but low-pathogenicity strains (which are naturally devoid of the HPI) can gain this lethal power if iron or an exogenous siderophore (Desferal) is administered to the animals [47]. Numerous clinical reports in humans have shown that low-pathogenicity strains of *Y. enterocolitica* (bioserotypes 4/O:3 and 2/O:9), usually responsible for moderate intestinal symptoms, can cause systemic infections in iron-overloaded patients. Thus, iron appears to play a crucial role in *Yersinia* pathogenesis. The reported suppressive effect of Desferal on the host immune response should however be taken into account when considering the dramatic effect of this compound. Nonetheless, it appears that in the absence of a high-affinity iron-chelating compound, pathogenic *Yersinia* only cause local symptoms of moderate intensity. Iron, provided exogenously (iron or Desferal treatment) or by the presence of an intrinsic high-affinity iron-chelating system (e.g. yersiniabactin) endows the bacteria with the ability to multiply in the host and to cause systemic infections.

Distribution and conservation of the HPI

Table 3 Effect of the mutation of specific HPI-borne genes on the virulence of *Yersinia*

Species (strain)	Mutated gene	Route of infection	LD_{50}		Ref.
			Wild type	Mutant	
Y. enterocolitica (WA)	*fyuA*	iv	5×10^4	$>1 \times 10^6$	45
Y. pestis (KIM5:Δ*psa*, *yopJ*–)	*psn*	sc	1.3×10^2	$>2.9 \times 10^5$	1
	irp2	sc	1.3×10^2	$>1.3 \times 10^6$	1
Y. pseudotuberculosis (IP2790)	*irp2*	sc	3.5×10^6	$>2.1 \times 10^9$	7
	irp2	iv	6.9×10^0	1.9×10^3	7
	irp2	ig	7.6×10^7	2.7×10^9	7

iv: intravenous, ig: intragastric, sc: subcutaneous.

Within the genus *Yersinia* The presence and size of the HPI within the *Y. pseudotuberculosis* species entirely correlates with serotype: a complete island is only found in strains of serotype I, a HPI with a 9 kb truncation in its left-hand part is characteristic of serotype III strains, and no HPI is detected in strains of other serotypes [3, 46]. Differences in the distribution of the HPI between subgroups of a single species are also seen for *Y. enterocolitica*, in which only strains of biotype 1B carry the island. In these strains, the 35 kb right-hand part of the HPI encoding the yersiniabactin machinery is well conserved whereas the left-hand part containing the cluster of repeated sequences is much less well conserved [6]. In *Y. pestis*, the HPI is present in all three biotypes and is always present in fresh isolates from diseased animals and humans [15].

Among other bacterial genera Although the HPI was initially detected in *Yersinia* spp., this pathogenicity island appears also to be present in other bacterial genera. The region extending from the *irp2* to the *fyuA* loci has been detected in pathogenic *Escherichia coli* [48]. It is most prevalent in septicemic (80%) and enteroaggregative (93%) *E. coli* (EAEC), but it is also present in enteropathogenic (5%) *E. coli* (EPEC), enteroinvasive (27%) *E. coli* (EIEC), and enterotoxigenic (5%) *E. coli* (ETEC). In contrast, the HPI has not been found in enterohemorrhagic *E. coli*, *Shigella* or *Salmonella enterica*. Curiously, a small proportion of the *E. coli* strains that contain an *irp2* locus lack the *fyuA* gene, a situation reminiscent of the HPI of serotype III *Y. pseudotuberculosis*, which also lacks the *fyuA/psn* region. A high degree of conservation of the HPI has been demonstrated by sequencing a region of the *irp2* and *fyuA* loci of *E. coli* which are more than 98% identical to those of the three highly pathogenic species of *Yersinia* [46, 48]. HPI genes and proteins have also been detected in other species of enterobacteria such as *Klebsiella pneumoniae, K. rhinoscleromatis, K. ozaenae, K. planticola, K. oxytoca* and *Citrobacter diversus* [13]. Interestingly, the G + C content of the *Klebsiella* genome (56%–57%) is close to that of the HPI, suggesting that these species may have been the bacterial donor of this element.

Conclusion

Like other pathogenicity islands, the HPI of *Yersinia* was probably acquired by integration of a bacteriophage genome (in this case a P4-like phage) into the bacterial chromosome. However, the HPI possesses two novel characteristics that have not been described for other pathogenicity islands. The first is the ability to integrate into any of the different chromosomal copies of the same tRNA gene and to be apparently able to "jump" from one of these genes to another on the same chromosome. The second is its wide distribution among various species of *Yersinia* and various members of the family *Enterobacteriaceae*. The high degree of sequence identity of HPI-borne genes between various *Yersinia* species and among other enterobacterial genera argues for the recent horizontal and independent acquisition of the same island by these different species. However, the HPI is present in all three *Yersinia* species, which have very different ecological niches, and its distribution is serotype-specific in *Y. pseudotuberculosis*, biotype-specific in *Y. enterocolitica*, and ubiquitous in *Y. pestis*, which is more suggestive of a single acquisition of the HPI by an ancestral *Yersinia* strain (maybe recently diverged from *E. coli*). Some strains may have lost their HPI during species evolution and have then differentiated into various biotypes and/or serotypes. Distinguishing between these two evolutionary hypotheses and investigating the intrinsic capacity of the HPI to spread among various microorganisms may provide important insight into the evolution of bacterial pathogenicity.

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