

Yersinia Pestis: Pathogenesis and evolution

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Bioinformatics

Abstract

Characterization of an unknown DNA fragment is important for understanding its pathogenesis and evolution. In this case study, a DNA fragment was analyzed and the organism of origin was determined to be ***Yersinia pestis***. It is the causative agent of plague, which evolved from *Yersinia pseudotuberculosis* ~5,000 years ago gaining increased virulence and transmission.

Introduction

In this study *Y.pestis* was identified from an unknown DNA fragment. Then, the Invasin pseudogene on this fragment was chosen to be studied further using literature research, gene annotation, multiple sequence alignment, a phylogenetic tree and analysis of the 3D structures.

The gram-negative nonmotile *Y.pestis* caused a total of 3 deadly plagues, including the black death killing around 1/3 of Europe (Barbieri, et al., 2020). The blood-borne disease normally occurs in rodents but could spread to humans by flea bites. According to experiments, *Y.pestis* descended from *Yersinia pseudotuberculosis*, an enteropathogen, around 5,000 years ago. Horizontal DNA transfer likely increased *Y. pestis*' virulence, therefore certain genes were no longer needed, like the Invasin gene. Those were lost (Parkhill, et al., 2001). Two adhesins are now expressed at increased levels; PsaA and the Ail protein, an outer membrane protein which mediates host cell attachment, provides resistance to the complement system of the host and plays an essential role in the administration of virulence factors into the host cells (Seabaugh & Anderson, 2024).

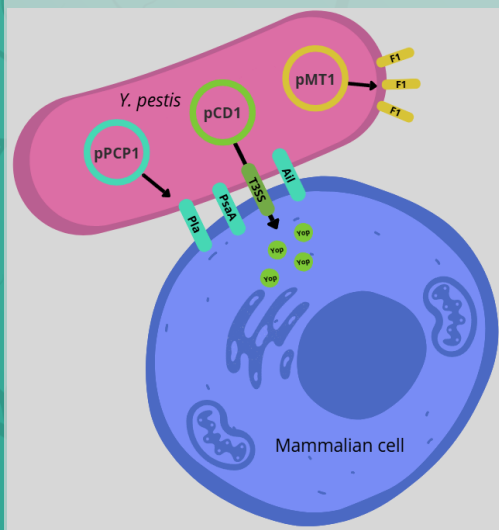


Figure 1. Pathogenesis of *Y. pestis*. Ail, PsaA and Pla are adhesins that keep the cell attached to the host and help evade the immune system. F1 forms small pili that aid in adherence and immune evasion. Through T3SS, Yops are injected that act on many pathways and establish an infection (Seabaugh & Anderson, 2024).

Materials and methods

BLASTN and ORFfinder:

Identification of *Y. pestis* via UniprotKB/Swiss-Prot and ClusteredNR database. To predict ORFs and identify genes via ORFfinder, these settings were used: Genetic code 11, start codon "ATG" and alternative initiation codons.

KBase and BLASTX:

Gene annotation by KBase database (Prokka) to identify genes and to confirm the Invasin gene and BLASTX against both the UniprotKB/Swiss-Prot and ClusteredNR database.

Multiple Sequence Alignment (MSA) and phylogenetics:

10 *Yersinia* species, including *Y. pestis*, selected from the ClusteredNR database and a Clustal Omega MSA was performed. Phylogenetic tree generated in iTOL (Mode = rectangular, Branch lengths = ignore)

3D protein structure analysis :

RCSB PSB was used to determine 3D structures of Invasin and Ail.

Results

Function	Start (nt)	Strand	Length (nt)
Protein YebF	325	+	411
Protease 2	1064	+	2052
Proline iminopeptidase	3837	+	951
DNA pol. III subunit theta	5077	-	231
Bacterial non-heme ferritin	5478	+	510
Protein YobA	6393	+	387
IMP YebZ	6782	+	885
Flagellar biosynthetic protein FlhB	9937	+	1161
Flagellar biosynthetic protein FlhA	11097	+	2079
Flagellar protein FlhE	13175	+	423
Invasin	14255	+	1011
Transposase IS200G	15255	+	459
Invasin	15803	+	2073
Flagella synthesis protein FlgN	18502	-	438
Negative regulator of flagellin synthesis	18826	-	303

Table 1. Results of Kbase. Annotation of the genes on the DNA fragment of *Y. pestis* using Kbase database. Hypothetical proteins were left out. Multiple Flagellar genes were located and two annotations of the Invasin gene.

Description	Scientific name	Percentage identity	E-value	Accession number
Invasin	<i>Y. pseudotuberculosis</i>	98.83%	0.0	P11922.2
Invasin	<i>Y. enterocolitica</i>	46.26%	1e-123	P19196.1
Intimin	<i>Escherichia coli</i>	32.10%	3e-39	P43261.2
Intimin	<i>E. coli</i>	32.51%	6e-39	O31000.1
Intimin	<i>E. coli</i>	31.60%	3e-38	P19809.2
Intimin	<i>Citrobacter freundii</i>	28.81%	1e-33	Q07591.1
Inverse autotransporter adhesin	<i>E. coli</i>	36.89%	2e-32	P76347.4

Table 2. Results of BLASTX. 6 best hits for ORF85, located via ORFfinder. Sorted by E-value.

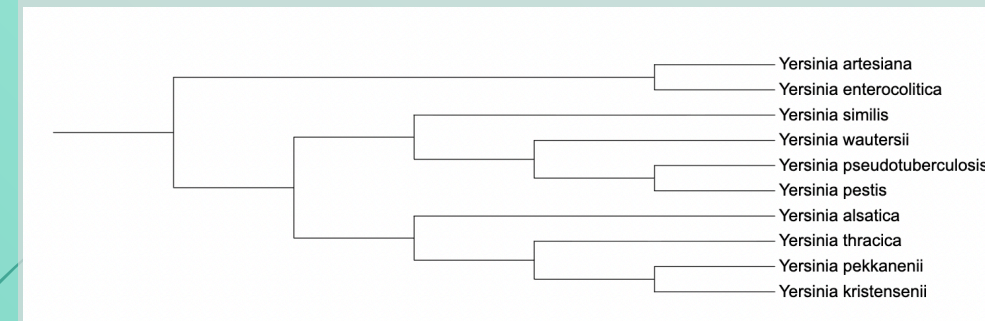


Figure 2. Phylogenetic tree of ten *Yersinia* strains containing the Invasin gene. Constructed with iTOL. *Y. pestis* is most closely related to *Y. pseudotuberculosis*.

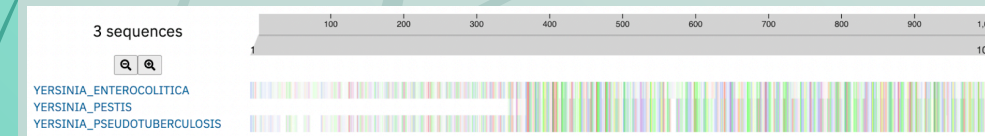


Figure 3. Clustal Omega Multiple Sequence Alignment of *Y. pestis* and two related strains with a working Invasin gene (1-1004 AA)

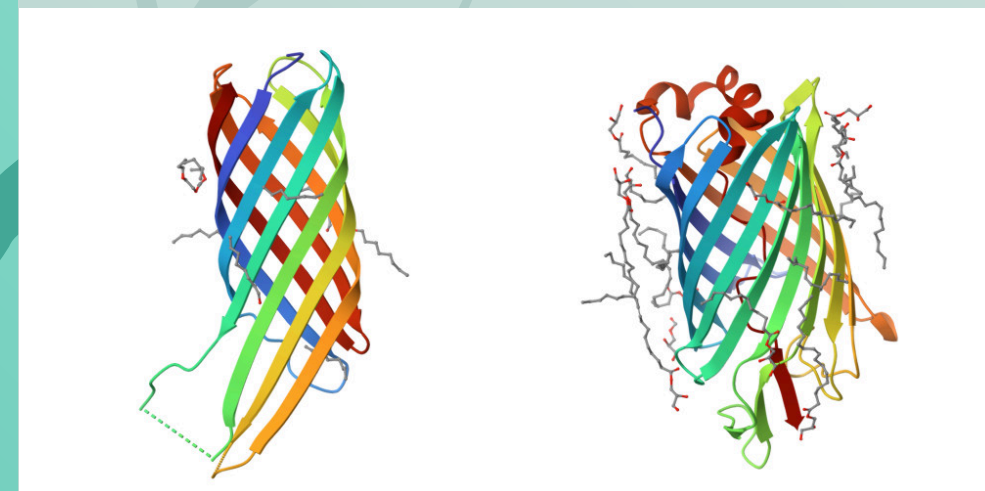


Figure 4. X-ray crystal structure of Ail protein β -barrel found in *Y. pestis* (left) and the X-ray crystal structure of Invasin from *Y. pseudotuberculosis* (right) derived from RCSB PDB

Conclusion

In this case study, we identified the Invasin pseudogene on the *Y. pestis* DNA fragment and revealed its similarity to Invasin in *Y. pseudotuberculosis* and other related species. Our gene annotation and phylogenetic analysis results showed that the Invasin gene in *Y. pestis* is disrupted due to an IS200G insertion, supporting the literature. The highly expressed Ail protein used in *Y. pestis*, which serves a similar function to Invasin, showed differences in 3D protein structure, explaining their different roles in adhesion and invasion. Together, these findings illustrate the virulence strategies in *Y. pestis*, specifically looking at the Invasin and Ail protein.

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