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# Translocated effectors of *Yersinia*

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## Summary

Currently, all known translocated effectors of *Yersinia* are delivered into host cells by type III secretion systems (T3SSs). Pathogenic *Yersinia* maintain the plasmid-encoded Ysc T3SS for the specific delivery of the well-studied Yop effectors. New horizons for effector biology have opened with the discovery of the Ysps of *Y. enterocolitica* Biovar 1B, which are translocated into host cells by the chromosome-endoded Ysa T3SS. The reported arsenal of effectors is likely to expand since genomic analysis has revealed gene-clusters in some *Yersinia* that code for other T3SSs. These efforts also revealed possible type VI secretion (T6S) systems, which may indicate translocation of effectors occurs by multiple mechanisms.

## Introduction

The genus *Yersinia* includes three human pathogens. The most infamous is the black death agent *Y. pestis*, which causes bubonic plague when it is transmitted by the bite of a flea and pneumonic plague when it is acquired through aerosol transmission [1]. In contrast, *Y. enterocolitica* and *Y. pseudotuberculosis* are enteropathogens transmitted by consumption of contaminated food or water [2]. These two organisms cause gastrointestinal syndromes that can develop into fatal septicemia in patients with compromised or underdeveloped immune systems. Regardless of the species and type of disease that ensues, translocation of toxic virulence effectors into host cells by type III secretion (T3S) systems plays an essential role in determining the outcome of a *Yersinia* infection. There are two recognized groups of effector proteins delivered by T3SSs among the *Yersinia*; the Yops and the Ysps.

## Yop effectors and the innate immune response

Interestingly, despite the various modes of transmission and diseases caused by the pathogenic *Yersinia*, they commonly have the plasmid-encoded Ysc T3SS for the delivery of six Yop effectors (YopE, YopH, YpkA/YopO, YopM , YopJ/P and YopT) (Table 1). Efforts to determine the biochemical activities of the Yops have revealed many targets are cellular components that influence the host innate immune response (Figure1). This arm of the immune system serves as the front line of defense against an invasive pathogen [3]. Macrophages and neutrophils are important elements of the early innate immune response and serve as sentries that take up and then inactivate invading bacteria. These cells, which act along with other facets of the innate immune response, are induced following the recognition of pathogen-associated molecular patterns (PAMPS) by the family of pattern-recognition receptors (PRRs) located on

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a variety of cell types. In response to PRR agonists or PAMPS, such as LPS or flagellin, signals are transduced and converged to activate multiple MAPKs and the NF-κB signaling pathways. This inflammatory response in turn leads to the production of numerous proinflammatory cytokines, including TNF-α, IL-12 and IL-18.

Given the important role that the MAPKs and NF-κB signaling pathways play in the earliest stages of the immune response, it is not surprising that one strategy of pathogenic *Yersinia* is to interfere with these essential signaling components [4-6]. It is well established that YopJ/P is a potent inhibitor of multiple NF-κB and MAPK pathways [7]. Recent studies have clarified the biochemical nature of YopJ/P activity by revealing it is an enzyme that acetylates serine and threonine residues in the activation loop of MKKs and IKKs [8-10]. This modification effectively blocks the residues from being phosphorylated and results in an inhibition of their activation thereby affecting cytokine production and inducing apoptosis of macrophages.

Phagocytosis of bacteria requires reorganization of the actin cytoskeleton through a process that is regulated by Rho family GTPases [11]. YopE is a Rho GTPase-activating protein (RhoGAP) which has been shown to stimulate GTP hydrolysis, thereby interfering with actin depolymerization mediated by the Rho family of proteins [12,13]. In addition to GTPase activation, it has been shown that membrane anchoring is also required for the activation of RhoA, Rac1 and Cdc42. Membrane anchoring occurs as the result of a post-translational modification resulting in C-terminal prenylation at a cysteine residue. YopT acts as a cysteine protease that removes this lipid modification through cleavage at a site a few residues upstream of the prenylated cysteine [14,15]. The resulting mislocalization inhibits normal signaling function [13,16].

Rho family members are further controlled by Rho guanine nucleotide dissociation inhibitors (RhoGDI). RhoGDI binds and sequesters inactive GDP-bound Rho family members primarily in the cytoplasm. YpkA/YopO contains a C-terminal domain that interacts with RhoA and Rac1 to mimic RhoGDI thereby preventing nucleotide exchange and activation [17]. Interestingly, YpkA/YopO also has an N-terminally located serine/threonine kinase domain that is activated by host cell derived actin, but its substrate remained a mystery for many years [18]. Importantly, a group of G proteins that respond to G protein coupled receptors have emerged as the YpkA/YopO kinase targets [19]. In a recent elegant study, it was demonstrated that YpkA/YopO specifically phosphorylates Ser47 of Gαq, a key residue located in the highly conserved diphosphate binding loop of the GTPase fold [19]. This phosphorylation impairs nucleotide binding and prevents Gαq-mediated cellular processes, including the subsequent activation of RhoA which would be expected to limit bacterial phagocytosis by cells. However, the exact role of YpkA/YopO kinase in affecting cellular activities remains an avenue open for further investigation since there are a myriad of signaling pathways affected by this Gαq group of G protein signaling molecules.

Phagocytosis is additionally inhibited by YopH, a protein tyrosine phosphatase that antagonizes several signaling pathways [20-22]. YopH specifically localizes to focal adhesion complexes where it interacts with and dephosphorylates proteins such as FAK, Cas and paxillin [21,23]. These proteins are involved in β1 integrin mediated phagocytic events brought about by the surface protein invasin of *Y. enterocolitica* and *Y. pseudotuberculosis* during epithelial cell invasion. Likewise, in macrophages, where uptake events can be mediated by complement receptors or Fc receptors, YopH dephosphorylates focal adhesin complex-associated proteins, including Cas, SKAP-HOM, Fyb and the FAK-homolog Pyk2 [24,25].

The remaining effector targeted by the Ysc T3SS is YopM, which has no clearly established function. YopM is known to traffic to the nucleus when introduced into HeLa cells. One study of the role that YopM plays during *Y. pestis* infection provides intriguing clues for future efforts.

YopM function correlates with the global depletion of natural killer cells by affecting expression of IL-15 and IL-15Rα [26].

## Ysp effectors of *Y. enterocolitica* Biovar 1B

While the Ysc T3SS is important for *Yersinia* virulence, it is now clear that some isolates of *Yersinia* utilize additional T3SSs to deliver virulence effectors into targeted host cells. The highly pathogenic *Y. enterocolitica* Biovar 1B carries the Ysa pathogenicity island (Ysa-PI) encoding a T3SS which is distinctly different from the Ysc T3SS and is more related to the Mxi-Spa T3SS of *Shigella* species [27-29]. The Ysa-PI is part of a larger region of the chromosome, called the plasticity zone, containing numerous other genes implicated in virulence [30].

Studies using the mouse model of Yersiniosis demonstrated that the Ysa T3SS plays an important role in *Y. enterocolitica* colonization of gastrointestinal tissues during the earliest stages of an infection [31]. This observation brings new attention to how enteropathogenic *Yersinia* colonizes the intestine and overcomes immune barriers presented by the host at this location. It may be that the Ysc T3SS is important for systemic stages of infection, while the Ysa T3SS is selective for gastrointestinal infection. Evaluating this model is dependent upon defining and delineating the function of the virulence effectors delivered by the Ysa T3SS.

The first proteins determined to be Ysps effector by virtue of their being substrates exported by the Ysa T3SS unexpectedly turned out to be YopE, YopN and YopJ/P, three proteins also exported by the Ysc T3SS [32] [27] (Table 1). As described above, YopE and YopJ/P are known to be effectors, but it is possible that YopN may also have an unrecognized effector function. It was further demonstrated that *Y. enterocolitica* Biovar 1B delivers YopJ/P into cultured macrophages and suppresses production of TNF-α by utilizing either the Ysa or Ysc pathways [32]. However, gaining a comprehensive understanding of how the Ysa T3SS mechanistically influences pathogenesis will depend on efforts to distinguish the functions of the effectors it delivers.

In this regard, eight additional translocated effectors have been identified (YspA, YspE, YspF, YspI, YspK, YspL, YspM and YspP) (Table 1). YspA was recognized from analysis of genes within the Ysa-PI [27]. Recently, a comprehensive proteomics approach defined the complexity of the Ysa T3SS secretome [33]. This effort confirmed YspA as an effector and additionally identified YspE, YspF, YspI, YspK, YspL and YspP. Each of these effectors was shown to be necessary for full virulence of *Y. enterocolitica* Biovar 1B in the mouse gastroenteritis competitive index assay [33]. Finally, YspM was identified later as an effector produced by a subset of Biovar 1B strains. When including YopE, YopN and YopJ/P, the Ysa T3SS appears to deliver a collection of ten, and potentially eleven, effectors depending upon the strain examined.

It is striking that the genes encoding the effectors delivered by the Ysa T3SS are dispersed throughout the genome [33] (Figure 2). One *ysp* is located within the Ysa-PI, three are located on plasmid pYV, but the seven remaining map to sites dispersed throughout the chromosome. The lack of *ysp* gene co-localization inspires the idea that the evolution of *Y. enterocolitica* Biovar 1B involved numerous genetic lateral transfer events. The dispersed gene distribution combined with the complexity of the Ysps suggests that *Y. enterocolitica* Biovar 1B has experienced strong selective pressure to maintain the Ysa T3SS. This may reflect how this group of *Yersina* has adapted to survive differently in the gastrointestinal environment and may help to explain the severity of human infections they cause.

With the exception of YopE and YopJ/P, the cellular targets of the effector proteins delivered by the Ysa T3SS are largely open for investigation (Table 1, and Figure1). Several of the Ysps

contain regions or domains that can be predicted to have particular functions. YspK serine/threonine kinase activity and YspP tyrosine phosphatase activity were demonstrated using *in vitro*-based biochemical assays [33]. Furthermore, YspK shares 91% identity with OspG, an effector produced by *Shigella flexneri* [34]. OspG can bind ubiquitinylated E2 conjugating enzymes, including UbcH5b that is responsible for targeting the degradation of many important signaling factors such as phospho-IκBα by the proteasome [34]. By interfering with phospho-IκBα degradation, its complex with NF-κB is maintained, which suppresses activation of this proinflammatory pathway. Using yeast two-hybrid analysis, YspK has also been shown to interact with E2 proteins (Matsumoto and Young, unpublished data). This result suggests YspK and OspG have similar functions in targeting cellular components to limit the innate immune response (Figure 1). YspM is predicted to be a GDSL lipase and when expressed in *Saccharomyces cerevisiae* it prevents cell growth. It is tantalizing to consider the predicted lipase activity may be targeting an essential cellular component but this remains to be demonstrated [35]. Other Ysps with interesting domains predicted by computational analysis include YspE with an ADP-ribosyltransferase motif (Matsumoto and Young, unpublished results). In other pathogenic bacteria ADP-ribosyltransferases are among the most potent virulence factors, marking YspE as a high priority for exploration of its cellular target [36]. Likewise, a calcium-binding motif predicted for YspI is a unique characteristic of an effector. This may indicate YspI belongs to a new class of virulence factors affecting cellular activities through a novel mechanism trigged by calcium sensing. Clearly these are speculative predictions, but they are presented to highlight the potential within the study of the Ysp effector proteins to reveal new facets of host-pathogen interactions.

## New *Yersinia* effectors yet to be discovered

Genomic sequencing of numerous strains of *Y. pestis* and *Y. pseudotuberculosis* have revealed a locus with the potential to encode a T3SS that is different than either the Ysc or Ysa T3SSs [37,38]. This system resembles the Ssa T3SS of *Salmonella enterica* [39]. It has also been reported that this locus is present in some *Y. enterocolitica* serotype O:3 strains [40]. To date, no clear link between this T3SS and virulence has been established. On another front, one controversial study has suggested the insecticidal toxin-like proteins (IT-like proteins) of *Y. pestis* and *Y. pseudotuberculosis* may form a new group of effectors [41]. This assertion was based on the observation that *in vitro* ectopic expression of these IT-like proteins led to secretion, and in one case even translocation into insect and mammalian cells, by the Ysc T3SS. The idea that the Ysc T3S is the native secretion pathway for the insecticidal toxin has recently been challenged, but it remains a formal possibility that they are exported by the chromosomal-encoded Ssa-like T3SS [42].

## Do *Yersinia* translocate effectors by other mechanisms?

Direct translocation of effectors into host cells by Gram-negative bacteria can additionally involve T4SSs and T6SSs. While no T4SS has been described, recent genomic analysis has suggested the presence of several potential T6SSs encoded by loci dispersed among *Y. pestis* and *Y. pseudotuberculosis* genomes [43,44]. Some of these gene-clusters could encode proteins homologous to the Hcp- and VgrG-families of secreted proteins and effectors. With the recent recognition that T6SS translocate effectors into host cells there appears to be abundant opportunity for new effectors to be discovered.

## Conclusions

The Yop effectors are nearly identical between *Y. pestis*, *Y. pseudotuberculosis* and *Y. enterocolitica*. The study of pathogenic *Yersinia* presents an opportunity to examine how these effectors are utilized by pathogenic species causing different types of infections. The discovery

of the Ysa T3SS of *Y. enterocolitica* Biovar 1B expands on this theme of comparative pathology by further revealing that some effectors, like YopE and YopJ/P, can contribute to disease when delivered through other pathways, such as the Ysa T3SS. However, the new twists on effector biology will come from further pursuing the functions of newly discovered effectors such as the Ysps. With the recognition of possible translocated effectors by T6SSs, there appears to be abundant opportunity for new effectors to be discovered.

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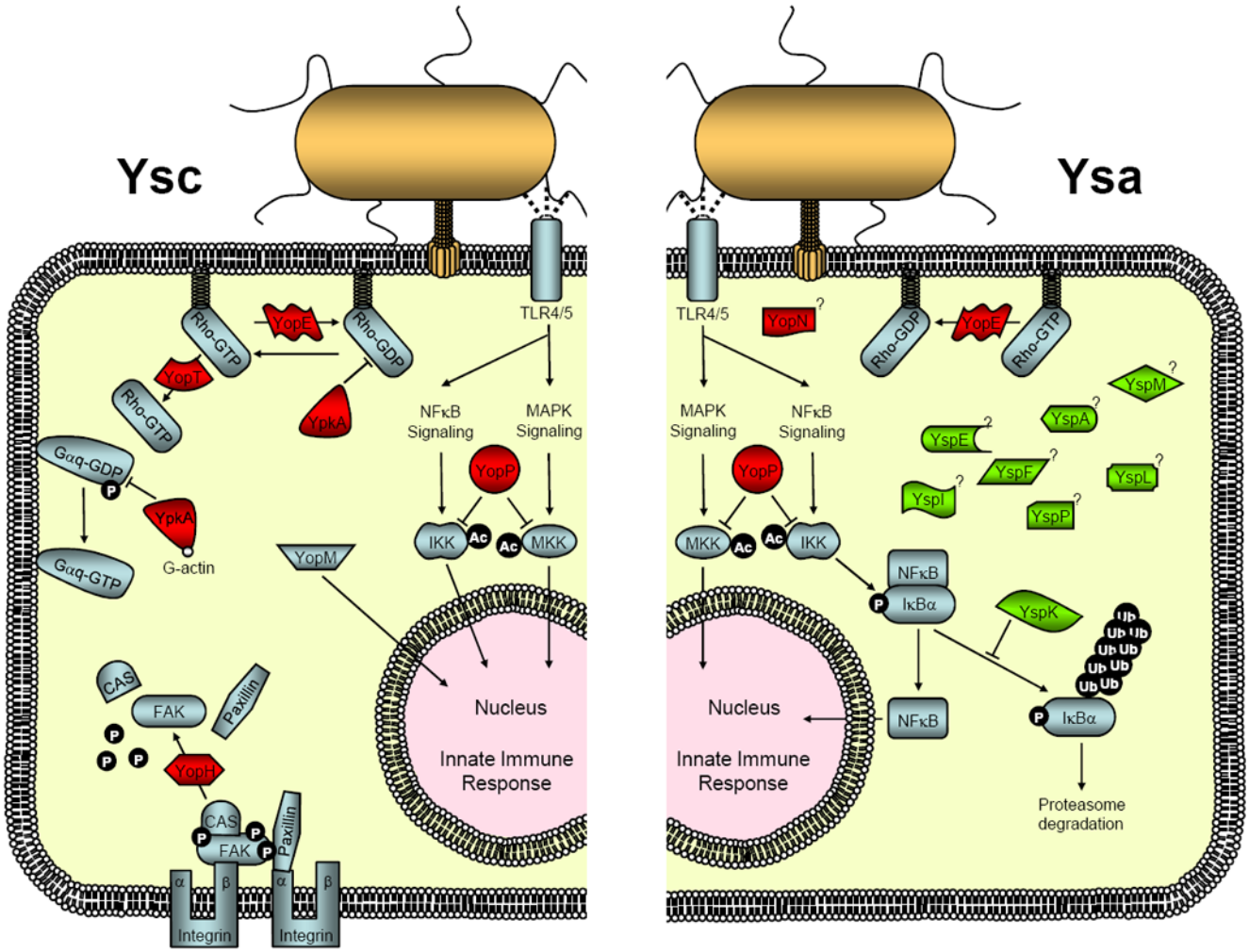


**Figure 1.**

Cellular targets and signaling pathways targeted by *Yersinia* effectors translocated by the Ysc and Ysa T3SSs. Left side - Effectors delivered by the Ysc T3SS found in *Y. pestis*, *Y. pseudotuberculosis* and *Y. enterocolitica*. Rho family GTPases are targeted by YopE, YpkA/YopO and YopT. The N-terminal serine/threonine kinase domain of YpkA/YopO phosphorylates Gαq to prevent activation of trimeric G proteins. YopH tyrosine phosphatase interacts and dephosphorylates proteins within focal adhesion complexes to inhibit phagocytosis. YopJ/P acetylates serine and threonine residues in the kinase activation loop of MAPKs and IKKs to prevent them from activation through phosphorylation. Inhibition of MAPKs and IKKs limits expression of proinflammatory genes. YopM traffics to the nucleus where it may affect gene transcription networks. Right side - Effectors delivered by the Ysa T3SS of *Y. enterocolitica* Biovar 1B. Presumably, YopE and YopJ/P function as described above. YopN is injected into cells but it does not have a defined effector function. YspK interacts with E2 ubiquitin-conjugating enzymes and may interfere with proteasome-mediated degradation of IκBα, negative factor controlling the activation of NF-kB thereby inhibiting the expression of proinflammatory genes.

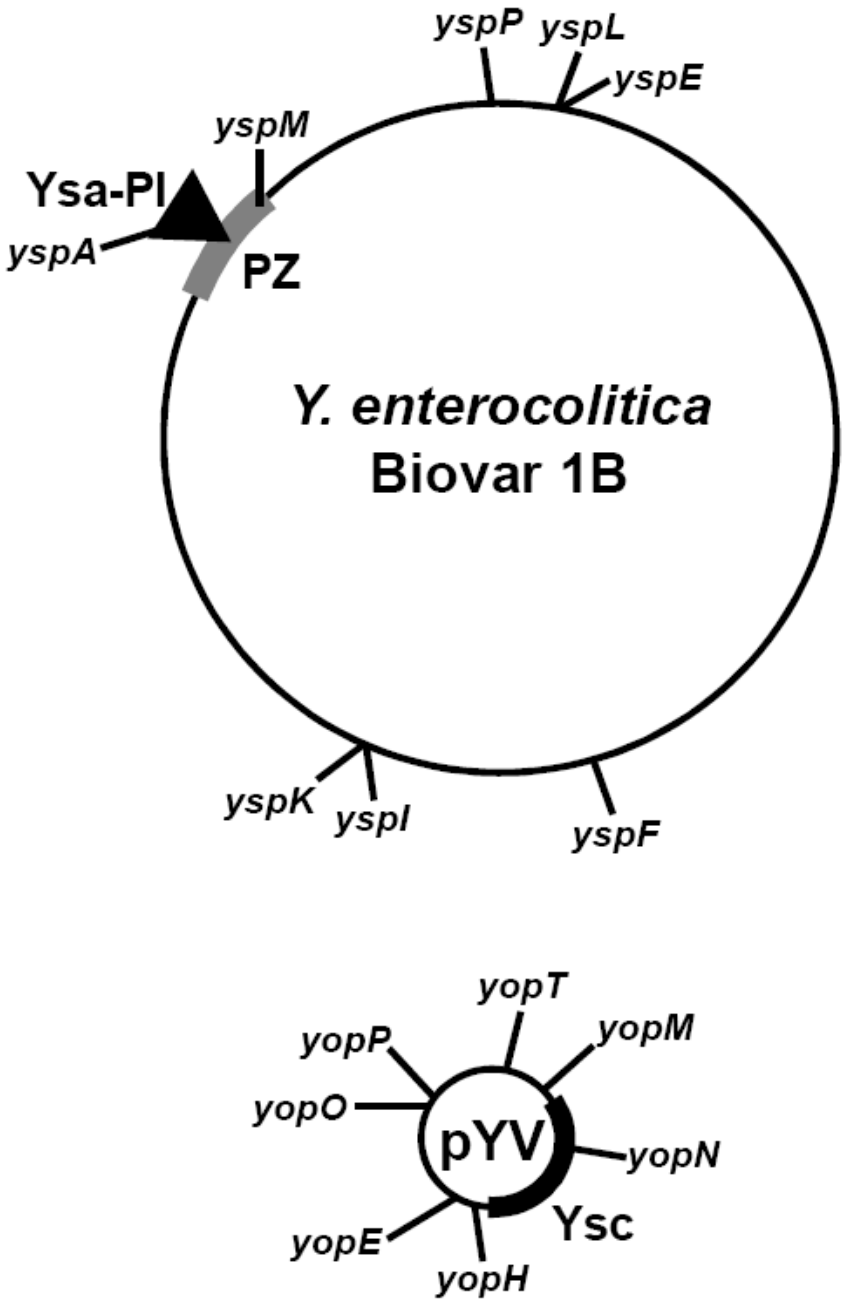


**Figure 2.**

Genes of *Y. enterocolitica* Biovar 1B that encode effectors translocated into host cells by the Ysa T3SS are dispersed in the genome. Depicted is the chromosome with labels indicating the relative locations of effector-encoding *ysp* genes and the location of the YSA-PI encoding the Ysa T3SS. The Ysa-PI is within a region of the chromosome rich with other genes implicated in virulence called the plasticity zone (PZ). Plasmid pYV carries genes encoding the Ysc T3SS (Ysc) and the effector Yops. A similar plasmid is present in *Y. pestis* and *Y. pseudotuberculosis*.

**Table 1**

Virulence effector proteins of pathogenic *Yersinia*

| Effectors | T3SS | Gene Locations | Biochemical Functions/Characteristics | Cellular Targets | Cellular Effects |
|---|---|---|---|---|---|
| **Pathogenic *Y. pestis, Y. psuedotuberculosis* and *Y. enterocolitica*** | | | | | |
| YopE | Ysc/Ysa | Plasmid | Rho GAP mimicry | RhoA, Rac1, Cdc42 | Disruption of actin cytoskeleton<br>Inhibition of phagocytosis |
| YopH | Ysc | Plasmid | Protein tyrosine phosphatase | Focal adhesion complexes ($p130^{Cas}$, FAK, paxillin, Fyb, SKAP-HOM, Crk, Pyk2)<br>Other proteins LAT, SLP-76, Lck | Disruption of actin cytoskeleton<br>Disruption of phagocytosis<br>Inhibition of chemoattractant protein (MCP1) production (P13K/Akt signaling)<br>Suppression of adaptive immune response (cytokine IL-2) |
| YpkA/YopO | Ysc | Plasmid | Serine/threonine kinase<br>RhoGDI mimicry<br>Actin binding | Gαq, RhoA, Rac1 Actin (kinase activity activation) | Disruption of actin cytoskeleton<br>Disruption of phagocytosis<br>Inhibition of Gαq signaling |
| YopM | Ysc | Plasmid | Localization to nucleus<br>Twelve to twenty tandem leucine-rich repeats (~20 amino-acid) | Rsk1 and Prk2? | Inhibition of cytokine production (IL-15 and IL-15Rα)<br>Depletion of NK cells |
| YopJ/P | Ysc/Ysa | Plasmid | Acetyltransferase | MAPKK and IKK family member proteins | Inhibition of MAPK and NF-κB signaling pathways<br>Suppression of proinflammatory cytokine and chemokine production (TNF-α, IL-8, IL-12, IL-18, etc.)<br>Induction of apoptosis |
| YopT | Ysc | Plasmid | Cysteine protease | RhoA, Rac1, Cdc42 | Disruption of actin cytoskeleton<br>Inhibition of phagocytosis |
| **Highly Pathogenicity *Y. enterocolitica* Biovar 1B** | | | | | |
| YspA | Ysa | Chromosome | ? | ? | ? |
| YspL | Ysa | Chromosome | Two separate 15 or 17 amino-acid repeats | ? | ? |
| YspP | Ysa | Chromosome | Protein tyrosine phosphatase | ? | ? |
| YspF | Ysa | Chromosome | ? | ? | ? |
| YspE | Ysa | Chromosome | ADP-ribosyltransferase? | ? | ? |
| YspI | Ysa | Chromosome | Ca2+ binding protein<br>Three tandem 12 amino-acid repeats | ? | ? |
| YspK | Ysa | Chromosome | Serine/threonine kinase | E2 ubiquitin-conjugating enzymes | Inhibition of NF-κB signaling pathway |

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| Effectors | T3SS | Gene Locations | Biochemical Functions/Characteristics | Cellular Targets | Cellular Effects |
|---|---|---|---|---|---|
| YspM | Ysa | Chromosome | GDSL lipase motif | ? | Growth inhibition of *Saccharomyces cerevisiae* |