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Yersinia spp. — The Search for YopX Function
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A recent proteomics study identified a new *Yersinia spp.* effector molecule Yersinia outer protein X (YopX). While functional questions to date have yet to be answered, conversation continues to follow suspicion YopX may in fact play a role in mitochondrial functions. The following approach, is a pragmatic structure towards solving the puzzle.

History has many overshadowing events when it comes to *Yersinia spp.* Although plague is a concern, it is not the most common affliction and only three of the seventeen *Yersinia spp.* are pathogenic to humans [1]. It is tempting to focus our energies on those species however a comparative analysis with non-*Yersinia spp.* may illuminate YopX activity in virulence. Currently, secreted Yop effectors are primarily known for virulence in conjunction with immune cells additionally requiring the use of both a type 3 secretion system (T3SS) and chaperone molecules [2]. As such, it is likely a non-pathogenic bacterium would have little use for such a protein. Because *Yersinia spp.* are well known for their evolutionary divergence when adapting to mammalian hosts, it is likely a bioinformatic search may shed light on the lacking presence of YopX in non-pathogenic organisms. Such presence would indicate YopX does not function in an immune related fashion. Because genomic and proteomic databases are not entirely complete, the lack of confirmation does not necessitate lack of YopX presence unless the entire Yop regulon has been confirmed absent. Conducting Multilocus Sequence Typing for obvious signs of divergence, followed by multiple sequence alignment and basic local alignment search tool (BLAST) of *Yersinia pestis, Yersinia enterocolitica,* related non-pathogenic *Yersinia entomophaga* which has been isolated in human urinary tract [3] as well as *Yersinia pekkanenii* which is lacking entire virulence plasmids and non-pathogenic to either humans nor their intermediates are reasonable comparisons to consider [4].

Having established YopX is limited to virulent forms or further mammalian specific pathogenesis, confirmation of T3SS into the cytoplasm will be required. While ideally YopX is a functioning component of less virulent *Yersinia spp.*, if necessary, plasmid construction and expression of *Y. pestis* in *Escherichia coli* has been successful [5] and will allow use of BSL-2 facilities rather than requiring a more specialized environment. Cya reporter assay with known necessary T3SS functional protein deficient mutants while in optimal growth conditions may be performed. Adenylate cyclase domain ($Cya_{2\text{-}400}$) of the *Bordetella pertussis* adenylate cyclase toxin (CyaA) is not capable of translocating itself, is only activated upon entry into the host cytoplasm and produces a distinct traceable signal [6]. Therefore, detection will identify if YopX is dependent on T3SS as are other Yop effectors. Further assay deleting Syc chaperones or Ysc secretion machinery may illude targeting, function or dependence for future consideration. Provided YopX is a T3SS dependent protein destined for host cytoplasm, targeting can be analyzed. It is important to note T3SS are not fully understood and calcium concentrations play a role in function [5]. Given the complexity of mitochondrial and endoplasmic reticulum functions in Ca^{2+} dependent roles, YopX may be reliant or affected by available Ca^{2+} or even play a role in acquisition thus requiring special attention. While STRING analysis does not yet predict YopX targeting, it is prudent to revisit at time of research due to connections such as yscX low Ca^{2+} response and other Yop effectors. MitoTracker Green FM can be a useful tool identifying protein localization to the mitochondria regardless of function. It's value here is mostly acting in differential targeting. It is important to remember *Yersinia spp.* virulence factors are both phasic and temperature dependent. Many functions are suppressed until physiological conditions are achieved such as nearing 37°C [5]. Using a reporter enzyme such as luciferase may be more appropriate. Size exclusion chromatography

may be useful for recovering YopX. Subsequent sequencing may illuminate chaperone molecules or binding partners. Provided localization occurs bioinformatic analysis using predictive software such as BSPRED or 3DLigandSite may also be useful identifying ligands and interaction potentials further. With identified binding partners, a single-cycle kinetic surface plasmon resonance experiment may be used to verify both binding and binding strength by measuring dissociation constants. If an interaction is confirmed a literature review of the YopX target may answer functional questions to remodel investigations. Although, it is just as likely to create more questions. RNAi screening with effector targets may be able to help *in vitro* confirmation but could be challenging given host specific preferential behavior.

At which point *in vivo* analysis has become appropriate, *Yersinia spp.* pathogenesis needs to be reconsidered. Biophotonic imaging with RNAi knockdown can track disease progression and provide evidence to whether YopX is a necessary during infection. Because *Yersinia spp*. infect a variety hosts and mammalian models require: additional resources, considerations and approval; using a flea or other intermediate host should be considered. It must be acknowledged, unique challenges in containment and immobilization during imaging present with a flea compared to rodent models. Amongst others, full mitochondrial gene sequencing exists for cat fleas and may provide interesting information to whether YopX is expressed specifically during human phases of infection rather than merely by the pathogen. Although this description does not exhaust all resources available, it does provide a construct for which identifying YopX function may begin. Wholly, YopX mitochondrial targeting potential presents a paradigm shift in this genus. Mitochondria functions include cell regulation and vacuolar trafficking. *Yersinia spp.* have been known utilize the host endosomal recycling pathway to advert detection by macrophages in a T3SS independent manner [7]. *Yersinia spp.* effector secretion from the vacuole until now have been recognized largely in regulation or evading immune response [2]. If YopX indeed targets the mitochondria we are seeing an active role controlling host cell machinery and new understanding in a genus' evolution that continues to lead the way.

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