สารพิษทีซีจำเพาะต่อสัตว์เลี้ยงลูกด้วยนมที่พบในแบคทีเรีย *Yersinia pestis* และ *Yersinia pseudotuberculosis*

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บทคัดย่อ

กลุ่มพิษขนาดใหญ่ (สารพิษทีซี) เป็นกลุ่มพิษที่มีโครงสร้างเป็นโปรตีนขนาดใหญ่ประกอบไปด้วยโครงสร้างขนาดย่อยสามส่วน โดยมีหลักการทำงานคล้ายกับเข็มฉีดยา มีช่องผ่านภายในหน่วยย่อยโปรตีน A และมีหน่วยโปรตีนย่อย B กับ C ประกอบกันอยู่ด้านบนของช่องผ่านภายในหน่วยย่อยโปรตีน A สารพิษทีซีสามารถพบได้ในแบคทีเรียก่อโรคในแมลงหลายชนิด โดยยังคงโครงสร้างและหลักการทำงานพื้นฐานที่พบครั้งแรกใน *Photorhabdus luminescens* ปัจจุบันได้มีข้อมูลจำนวนหนึ่งที่บ่งบอกถึงสารพิษทีซีลักษณะแปลกแยกออกไปใน *Yersinia pestis* และ *Yersinia pseudotuberculosis* สารพิษทีซีลักษณะคล้ายกลุ่มฆ่าแมลงเหล่านี้แสดงฤทธิ์ฆ่าแมลงที่ลดลงและฤทธิ์จำเพาะต่อสัตว์เลี้ยงลูกด้วยนม โดยเฉพาะสารพิษทีซีใน *Y. pestis* ที่ต่างออกไปอย่างชัดเจนเช่นถูกกระตุ้นการแสดงออกเป็นพิเศษในพาหะหมัด พิษที่ไม่มีฤทธิ์ต่อหมัดและความสามารถในการหยุดยั้งฟาโกไซโตซิสทันทีหลังจากการติดเชื้อ หากสามารถสรุปได้ว่าสารพิษทีซีใน *Y. pestis* ถูกคัดหลั่งโดยไม่พึ่ง T3SS (type III secretion system) ข้อมูลที่มีอยู่ในปัจจุบันเสนอว่าสารพิษทีซีใน *Y. pestis* อาจจะมีหน้าที่ที่ช่วยยับยั้งฟาโกไซโตซิสในช่วงปรับอุณหภูมิของ T3SS

คำสำคัญ: สารพิษทีซี ลักษณะคล้ายกลุ่มฆ่าแมลง จำเพาะต่อสัตว์เลี้ยงลูกด้วยนม *Yersinia pestis Yersinia pseudotuberculosis*

รับบทความ: *25 มกราคม 2563* ***แก้ไข:*** *9 มีนาคม 2563* ***ตอบรับ:*** *3 พฤษภาคม 2563*

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Mammalian-Specificity in TC Toxins Found in *Yersinia pestis* and *Yersinia pseudotuberculosis*

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Abstract

Toxin complex family proteins (TC toxins) are a family of large, tripartite, high molecular weight, traditionally insecticidal toxins operating through a syringe-like mechanism; comprised of a bell-shaped subunit A and a chamber formed from subunit B and C situated above a translocation channel within subunit A. First identified in *Photorhabdus luminescens*, homologs of TC toxins can be found within several insect pathogen bacteria, all sharing similar structure and mechanism. In recent years, evidence of an insecticidal-like form of TC toxin with mammalian-specific toxicity has emerged in *Yersinia pseudotuberculosis* and *Yersinia pestis*. These insecticidal-like deviations of TC toxins exhibit reduction in insect toxicity and display toxicity towards mammalian cells. Within *Y. pestis*, TC toxin has gained further distinct qualities such as preferential gene upregulation in flea vectors, null toxicity towards flea vectors, lack of contribution to transmission quality, and inhibition of phagocytosis immediately following transmission. If *Y. pestis* TC toxin T3SS (type III secretion system) independence can be conclusively determined, the current data suggests *Y. pestis* TC toxin may serve as an interval bridge providing phagocytosis resistance prior to the T3SS temperature-shift process.

Keywords: TC toxin, Insecticidal-like, Mammalian-specificity, *Yersinia pestis*, *Yersinia pseudotuberculosis*

Received: *25 January 2020,* ***Revised:*** *9 March 2020,* ***Accepted:*** *3 May 2020*

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Introduction

Among bacterial pathogens, there exist an extremely diverse and varied multitude of virulence factors all with its own individual mechanisms and pathogenesis towards its host, ranging in quality such as specificity and severity. A number of notable virulence factors are commonly bacterial toxins[1], classified into exotoxin and endotoxin, especially exotoxin critical importance towards pathogenic bacteria[2, 3]. In the midst of bacteria exotoxins are the Toxin Complex Family proteins or TC toxins, first garnering attention within *Photorhabdus luminescens* as an alternative insecticidal agent to combat *Bacillus thuringiensis* toxins resistance[4], the TC toxin family and its homologues later were found and reported in an extensive number of bacteria, primarily in *Enterobacteriaceae*[5]. Most notable microbes include *P. luminescens* and *Serratia entomophila*, and these TC toxins exhibit insecticidal qualities[4, 6]. This ubiquity of TC toxins extends to the *Yersinia* spp., although it was within *Yersinia pestis* and *Yersinia pseudotuberculosis* that toxicity towards mammalian cells was discovered[7]. Along with a reduction in insecticidal activity in both *Yersinia* TC toxins, the *Y. pestis* TC toxin apparent contribution towards resistance of host immune response suggests its defensive role against host's innate immunity following immediate introduction[8, 9]. Interestingly, only *Y. pestis* and *Y. pseudotuberculosis* TC toxins were subjected to reduction in insecticidal activity, while homologs found in other *Yersinia* spp. still retain insecticidal activity and toxicity[10-12].

Given this information, this review focuses on the much less emphasized TC toxins, both the insecticidal form of TC toxins found in microbes currently identified as biological pesticides such as *P. luminescens*, and the presumed mammalian-adapted insecticidal-like form of TC toxins found in *Y. pestis* and *Y. pseudotuberculosis*.

Structure and mechanisms of TC toxins

TC toxins or toxin complex family proteins were first discovered in *P. luminescens*, and following extensive studies, the TC toxin found in *P. luminescens* was considered the best character for studying TC toxin homologues found in other species[4, 13, 14]. This *P. luminescens* TC toxin was found to be active against *Manduca sexta* and insects from several orders such as Lepidoptera, Coleoptera and Dictyoptera[4]. TC toxins are large, enzymatic, pore-forming, complex tripartite holotoxins comprised of three toxin subunits: TcA, TcB and TcC (Figure 1)[13]. Unlike other pore-forming toxins, TC toxins operate through a unique syringe-

like mechanism that involves injection of cytotoxic enzyme into host cells through a system that depends on the entire toxin's structure as a whole[14]. Although as a noteworthy point, TcA alone does display sufficient toxicity in the absence of TcB or TcC regardless of the complete complex requirement to exhibit full toxicity[15].

The largest subunit, the structure of the TcA subunit (*P. luminescens* TcdA1), resembles a pentameric bell with a translocation channel located within the protein structure[13, 14]. TcA is the subunit that binds to host cell membranes, forming four receptor binding sites, while the translocation channel within the subunit acts as the syringe that penetrates host cell membranes, transporting the cytotoxic enzyme into the host (Figure 2)[13]. In its inactivated prepore state, the translocation channel remains sheathed within TcA, only when the toxin was subjected to endosomal acidification would the translocation channel be pushed out by an entropic spring, transitioning into the activated pore state through a pH change[13, 14].

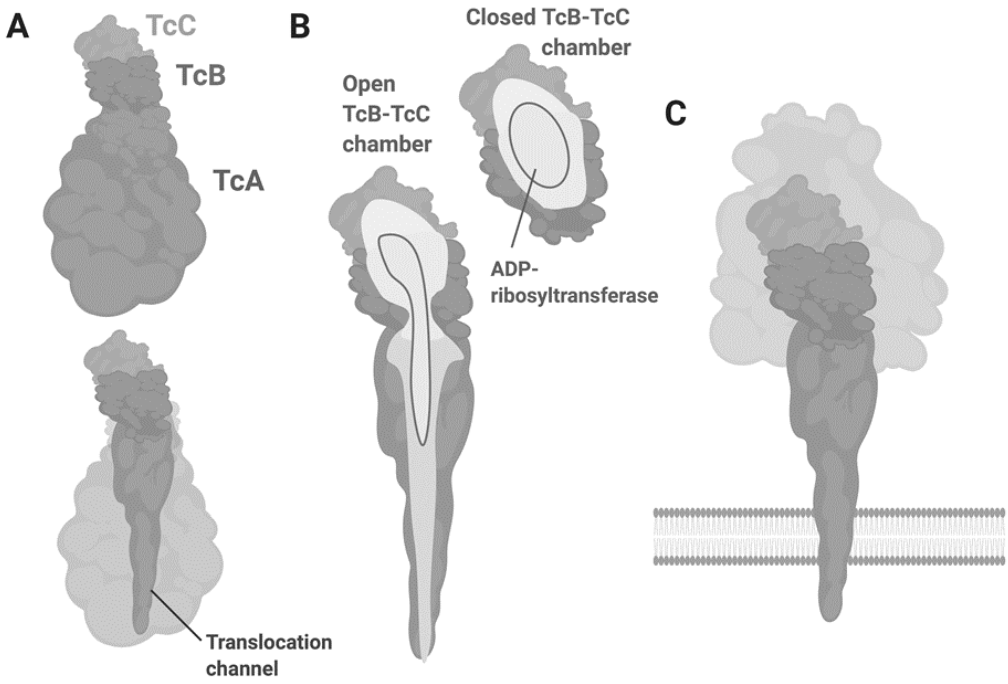

Figure 1. Simplified illustration model of the general TC toxin structure. (A) TC toxin in prepore state. Translocation channel is sheathed within TcA and connected to TcB-TcC. (B) The closed TcB-TcC chamber contains ADP-ribosyltransferase. Binding of TcA to TcB-TcC opens the chamber and allows continuous translocation of ADP-ribosyltransferase into the translocation channel. (C) TC toxin in pore state. Injection of the translocation channel perforates the membrane. Created with BioRender.com

The other two subunits, TcB and TcC, are significantly more compact structures which are located on top of the translocation channel of TcA. In their natural holotoxin forms, TcB and TcC work together as a chamber containing the aforementioned cytotoxic enzyme identified as an ADP-ribosyltransferase, derived from autoproteolytic cleaving of the cytotoxic C-terminal region of TcC[16, 17]. Intracellular exposure to ADP-ribosyltransferase results in agglutination of actin and activation of RhoA proteins, leading to forced agglutination and clustering of actin cytoskeleton which can inhibit phagocytosis[18]. The change in pH destabilize an electrostatic lock in TcA, which triggers injection of the translocation channel, releasing ADP-ribosyltransferase into host endosomal membrane[14, 19], Upon binding to TcA in prepore state, TcB-TcC binds to TcA translocation channel, inducing structural changes to TcB six-bladed β-propeller, a 'gatekeeper' located at the binding domain between TcB and TcA[19]. This structural change widens the inner diameter of the six-bladed β-propeller, creating a continuous translocation channel between TcB-TcC and TcA, allowing the cytotoxic enzyme to be released from the chamber.

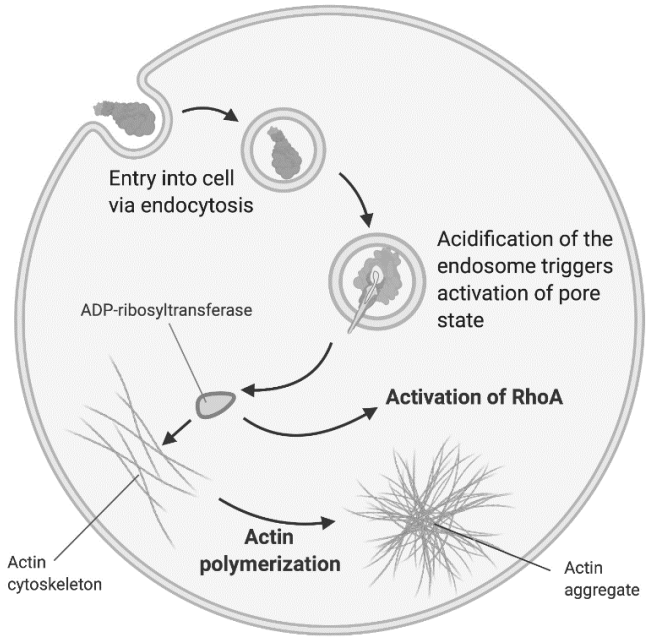

Figure 2. TC toxin mechanisms of cytotoxicity. The prepore to pore state transition of TC toxin is dependent on pH. Acidification of the endosome triggers the pore state. The released ADP-ribosyltransferase will then go on to cause actin polymerization and consequently clustering of the actin cytoskeleton, along with activation of RhoA proteins. Created with BioRender.com

Presence of TC toxins in other microbes

Homologs of TC toxins have been found mostly in various *Enterobacteriaceae* members, each varying in their resemblance to TC toxins found in *P. luminescens*, although TC toxins have also been reported in other gram-negative bacteria, *Pseudomonadaceae*, and specific gram-positive bacteria[5, 20, 21]. As of date, structural information on TC toxin homologs are limited in less studied species, and without structural research, most TC toxins are presumed to possess A, B, and C protein complexes that could vary in shape and size but still retain basic functions established in *P. luminescens*. It may be assumed that the presence of TC toxin gene among microbes is perhaps transferred either vertically or horizontally through evolutionary events[7, 10, 22]. Yet, more evidence is needed.

As described previously, for a toxin complex to be fully assembled and achieve full toxicity, all three subunits must be present[6, 15, 31]. Outside of *P. luminescens*, homologues of TC loci are responsible for synthesizing proteins into a toxin complex. These TC gene homologues mostly operate through the same system, with similar genes coding only for certain subunits; A subunit (*tcaAB*/*tcdA*-like genes), B subunit (*tcaC*/*tcdB*-like genes), and C subunit (*tccC*-like genes)[4, 23], along with the complete complex most likely retaining the same injection mechanism as well as conserving the function of each subunit to resemble the original function found in *P. luminescens* (Table 1)[13]. Sep proteins found in *Serratia entomophila* is one instance of TC proteins homologs that have yet to be characterized of their molecular mechanism and structure - though striking similarity was found between SepA with TcbA/TcdA/TcaB/TccB, SepB with TcaC, and SepC with TccC[6]. Together with clear correlation and causation link between Sep proteins and gut clearance symptoms of amber disease in *Costelytra zealandica*[24], it is plausible Sep proteins structure and mechanism potentially mirror those of TC proteins in *P. luminescens* due to their functional and genetic similarity[6].

In other well-studied insect pathogen species such as *Xenorhabdus nematophila* and *Yersinia entomophaga*, the same common architecture and pore formation mechanism are observed, with differences in non-structural features such as receptor-binding domains (RBDs) and neuraminidase-like domain in their respective toxin complexes[25], likely as a result of evolution and its ubiquitous nature. Complete genome sequencing of *Xenorhabdus nematophila* strain ATCC 19061 and *Xenorhabdus bovienii* strain SS-2004, when compared to the whole genome of *P. luminescens* strain TT01[32], suggested a common ancestor once existed between

Photorhabdus and *Xenorhabdus* that may be capable of residing in the two genera respective symbiotic nematode hosts, *Heterorhabditis* and *Steinernema*, due to their phylogenetic similarity[22]. This similarity can be observed in *X. nematophilus* toxin complex which is composed of three parts; XptA1/XptA2, XptB1, and XptC1 proteins. The genes *xptA1*, *xptB1*, and *xptC2* express toxicity towards a variety of insects such as *Pieris rapae* and *Pieris brassicae*, whereas *xptA2*, *xptB1*, and *xptC1* expression were much more limited, with activity only observed in certain species, such as in *Heliothis virescens*[30]. *X. nematophilus* achieves this alternation through single polycistronic expression of *xptA2*, *xptC1*, and *xptB1*, while *xptA1* is independently expressed[30]. Both XptA1 and XptA2 forms a tetramer structure from multiple protein monomers[26, 27]. It should be noted that a recent study showed that the structure of XptA1 is a bell-shaped pentameric complex similar to *P. luminescens* TcdA1, with around 46.7% amino acid sequence identity between the two complexes, instead of a tetramer. Hence, a pentameric structure as compared to a tetramer is possible[25]. Another well studied species is *Y. entomophaga*, with the TC homologue Yen-TC, comprising of TcA, TcB, TcC components as well as chitinases Chi1 and Chi2 proteins. The A1 and A2 subunits and Chi1 and Chi2 proteins in Yen-Tc of *Y. entomophaga* give rise to a pentameric complex similar to TcdA1[12, 33]. Yen-Tc is also the first account of TC toxin to show endochitinase activity as well, following the presence of Chi1 and Chi2 proteins which enables chitinolytic activity[34].

TC toxins are not ubiquitous in merely insecticidal bacteria, but in other microbes that are not inherently associated with insect as well. Examples include toxin complex genes homologues that were described in *Vibrio parahaemolyticus* strain 13-028/A3 within a genomic island[35], however, given that *V. parahaemolyticus* thrive in marine environments and is responsible for the widespread marine infection in shrimp as the causative agent of acute hepatopancreatic necrosis disease, it is unknown whether *V. parahaemolyticus* TC toxin toxicity extends to or has adapted to marine animals or not[35]. Likewise with the prevalence of TC toxins in *Enterobacteriaceae*, TC toxin homologs have also been reported in the family *Pseudomonadaceae*. *P. taiwanensis*[20], and four subgroups of *P. fluorescens* are examples of *Pseudomonadaceae* found with Toxin Complex gene clusters[21].

Given the universal nature of TC toxins, it is not surprising that TC toxins can be found in a large portion of members of *Enterobacteriaceae*. However, it is within the *Yersinia* spp. that the function

and role of TC toxin starts to significantly diverge from the toxin's previously established insecticidal role. TC toxins have been found in all three human pathogenic *Yersinia* spp., which are *Y. pestis*, *Y. pseudotuberculosis* and *Yersinia enterocolitica*. Interestingly, among the three, only homologs of TC genes in *Y. enterocolitica* are uncommonly distributed between certain strains and biovars, exhibiting oral toxicity against *M. sexta* larvae, with high TC genes expression following low temperature (10–20°C) incubation and minimal TC genes expression following body temperature incubation[11, 28]. Additionally, *Y. enterocolitica* strain T83 biotype 1A mutants lacking tcbA, tcaC, and tccC genes exhibited reduced ability to thrive in mice gastrointestinal tracts, suggesting the first example of TC toxins having non-insecticidal functions and playing a role in mammalian host colonization[36].

Table 1. Notable members of insecticidal and insecticidal-like types of TC toxin present in selected microorganisms.

Toxin Classification	Protein Homologs	Organism	Reference
Insecticidal	TcA (*tcdA1*, *tcaA1*, *tcaB1*, *tcbA*, *tccA*, *tccB*), TcB (*tcdB1*, *tcaC1*), and TcC (*tccC1*, *TccC2*) proteins	*Photorhabdus luminescens*	4, 23
	SepA, SepB, and SepC proteins	*Serratia entomophila*	6, 24
	XptA1, XptB1, XptC1 and XptA2, XptB1, XptC1 proteins	*Xenorhabdus nematophilus*	26, 27
	A1, A2, B, C, Chi1 and Chi2 proteins	*Yersinia entomophaga*	12
	A (*tcaA*, *tcaB1*, *tcaB2*), B (*tcaC*), and C (*tccC*) proteins	*Yersinia enterocolitica**	11, 28
Insecticidal-like	YitA, YitB, YitC, YipA, and YipB proteins	*Yersinia pestis*	29
	tcaA, *tcaB*, *tcaC*, and *tccC* gene product protein	*Yersinia pseudotuberculosis*	10

This table is far from comprehensive as other organisms with TC toxin does exist. However, research on their structure and function is limited/varied significantly across species/strain, and thus details is limited. *= TC toxin found only in certain strains/biovars.

Insecticidal-like TC toxin

In terms of structure, limited information exists on *Y. pestis* insecticidal-like TC toxin stoichiometry. As for *Y. pseudotuberculosis*, a recent paper by Leidreiter et al. (2019) had elucidated the stoichiometry of *Y. pseudotuberculosis* TcA in extensive detail[25]. The study had reported similar architecture and mechanisms in TcAs of *P. luminescens* (TcdA1 and tcdA4), *X. nematophila*, *Morganella morganii*, and *Y. pseudotuberculosis*, and proposed all existing TcAs to most likely share the same common architecture and mechanism of a pentameric bell-shaped structure surrounding a central pore-forming channel. However in between all five TcAs of the study, *Y. pseudotuberculosis* TcA was the one with the most structural difference, having been composed of two subunits; tcaA and tcaB, instead of a single protein. *Y. pseudotuberculosis* TcA also took a slimmer and more cylindrical shape than that of other TcAs, with tcaA forming the α-helical shell and tcaB forming the pore-forming domain and the lower part of the toxin structure. Of most distinct deviations in *Y. pseudotuberculosis* TcA is the lack of RBDs found on other TcAs which might reflect an adaptation of host specificity and partial closure at the bottom of the shell formed by the neuraminidase-like domain. There is also an additional presence of an extra coiled-coil domain reaching out to the TcB-binding domain of TcA and an enlarged loop belonging to the α-helical shell. Removal of this extra coiled-coil domain results in less stability and a higher chance of disassembly, suggesting this domain's function is to compensate for the decreased stability the absence of RBDs gave rise to. Of note, Leidreiter et al. also suggested *M. morganii* TcA might play a role in the bacterium pathogenic effects due to the subunit active functionality and periphery structural difference, such as within the RBDs which indicate adaptation to different host cells. However, research into TC toxin of *M. morganii* is limited and thus will be omitted from this review. In the case of *Y. pestis*, as a recently deviated clone of *Y. pseudotuberculosis* emerging in the past 1,500-20,000 years[37], *Y. pestis* retained at least 97% nucleotide identity in 75% of its chromosomal genes[38]. This genetic similarity gives rise to a high likelihood for similarity between the two species TC toxins.

Significant deviation in TC toxin role as an insecticidal protein can be found in *Y. pestis* and *Y. pseudotuberculosis*, unlike *Y. enterocolitica* which retained insecticidal toxicity. In contrast to *Y. enterocolitica* TC gene homologues, the respective TC loci in *Y. pseudotuberculosis* and *Y. pestis* are shared between all strains

within the same species, although variation can exist in certain genes between individual strains, such as in the *tcaC* gene[7, 29]. Genome comparison between *Y. pestis* and *Y. pseudotuberculosis* revealed the presence of TC homologues, with *Y. pseudotuberculosis* tcaA appearing highly conserved, *tcaB* possessing highly similar sequence to *Y. pestis* strain CO92 only in a limited number of strains, and *tcaC* appearing much shorter in *Y. pseudotuberculosis*[38, 39]. TC protein homologues in *Y. pestis* are titled YitA (TcaA-like) and YitB (TcaB-like), YitC (TcaC-like), and YipA and YipB (both TccC-like), with an upstream regulator *yitR* on the insecticidal-like toxin locus, which if overexpressed at low temperature, a large intracellular protein complex can be observed within insect and mammalian cells[40], or at the very least, YitA and YipA[29]. The complete protein complex product is composed of the five encoded protein components[40]. YipA and YipB is also reported to share amino acid similarity to TcC proteins of *P. luminescens*, and Yip A contains a putative protein tyrosine phosphatase, similar to the one belonging to *Y. pestis* effector protein YopH[8]. As of the writing of this literature, the mode of insecticidal-like TC toxin expression is still unclear, and contradicting data exists whether TC proteins are secreted into host cells through type III secretion system (T3SS) or TC proteins are naturally present on the surface of *Y. pestis*[8, 29, 40].

It has been shown that TC toxin in *Y. pseudotuberculosis* has lost remarkable lethality in insect hosts. Comparison of *Y. pseudotuberculosis* TC toxin with *P. luminescens* TC toxin showed lack of strong potentiation found in *P. luminescens,* demonstrating reduced toxicity in *Y. pseudotuberculosis* TC toxin towards *M. sexta* larvae[10]. Inoculation of TC genes knock-out mutants of *Y. pseudotuberculosis* into fleas also produce the same level of toxicity as non-mutants, which is contrary to the expected loss of toxicity in general due to the loss of TC genes[41]. In light of reduced insecticidal toxicity observed in *Y. pseudotuberculosis,* mammalian toxicity was investigated as a possible function. According to Hares et al. (2007), *Y. pseudotuberculosis* lysates containing A and BC components showed actin rearrangement as well as nuclear fragmentation in Caco-2 cells, especially if A and BC components are combined in advance, which could suggest structural similarity to traditional syringe-like TC toxins that rely on the same injection mechanism enabled by the complete toxin complex[13]. The researchers further reported that *Y. pseudotuberculosis* lysates did not exhibit similar effects in NIH3T3 cells, a cell line of immortalized murine fibroblasts. Instead, *Y. pestis* lysates

containing A and BC components caused significant actin condensation and nuclear fragmentation in NIH3T3 cells, though notably *P. luminescens* cell lysates containing A and BC components also exhibit the same effect. This could suggest general toxicity rather than insect-specific toxicity in TC proteins. Hence, it is possible that there is evolutionary transition from general toxicity to mammalian-specific toxicity as opposed to the huge evolutionary jump from insect-specific toxicity to mammalian-specific toxicity.

This putative possible mammalian role becomes more pronounced in *Y. pestis*. As a zoonotic disease with flea vectors, *Y. pestis* is well known to not exhibit toxicity in *Xenopsylla cheopis*. However infected fleas do eventually die, presumably from starvation due to fleas' proventriculus biofilm blockage rather than any direct toxicity[7, 41, 42]. A variety of TC genes were shown to be expressed and upregulated within fleas. Of note are *Y. pestis* TC genes *tcaA1*, *tcbA*, and *tcaC1*. Other than within fleas, they were reportedly upregulated within murine macrophages-like cells J774.1 as well[43]. Although information on the possible function of upregulation is unclear, it could be inferred that this upregulation should contribute in some manner to survival within macrophages, and that this function would likely not impact solely fleas. In addition, *Y. pestis* YitA and YipA proteins were shown to be preferentially expressed in fleas compared to *Y. pestis* grown in vitro at 21°C, or minimal expression found at 37°C, regulation decreasing down to 3-fold in *yitA* and 4.2-fold in *yitR*[29]. Interestingly, unless there is an overexpression of *yitR,* YitA and YipA proteins are not synthesized *in vitro*[29]. Moreover, both *yit* and *yip* genes are both upregulated within fleas, but not in rat buboes. These insecticidal-like TC protein homologues could be upregulated anywhere from 4 to 50 folds in the flea midgut. Deletion of *yitR* resulted in a major reduction in phagocytosis resistance post-transmission in *Y. pestis*, whereas expression of both *yit* and *yip* genes incurred no statistically significant difference in transmission[9, 29]. The suggested role of *Y. pestis* TC toxin as transmission factors has been disproven on more than one occasion, all of which resulted in no significant difference in flea bites or quality of transmission[9, 29]. Instead, transit through flea vector appears to enhance survival during the transitional period between host invasion and activation of temperature-regulated T3SS, which upregulate other virulence factors[8, 44]. *Y. pestis* initial post-transmission resistance against phagocytosis from murine bone marrow-derived macrophages and murine polymorphonuclear leukocytes (PMN) suggests that if the TC proteins are located

on the surface of *Y. pestis* (YipA and the presumably surface-exposed YitA), it might play a part in inhibition of phagocytosis during the period of post-transmission vulnerability during the temperature-shift process of T3SS to 37°C, rather than exposing *Y. pestis* to host immune responses unequipped immediately post-transmission[8, 9, 29] .

However, the research in this area is extremely limited, particularly with regard to the possible TTSS dependence of insecticidal-like TC toxin in *Y. pestis*. Both hypotheses cannot be considered conclusive argument for the mammalian function of insecticidal-like TC toxins, and additional research is needed to draw a reliable conclusion. This literature only serves as an insight into current hypotheses, and does not provide concrete evidence without further studies.

Conclusion

TC toxins are a growing family of high molecular weight complex toxins that can be found in a variety of bacteria, from established insecticidal bacteria to bacteria that may not be inherently associated with insecticidal activity nor a major role in insect pathogenesis. Despite sharing a highly similar basic structure and injection mechanism found in *P. luminescens*, the ubiquity in TC toxins among bacteria with these toxin complexes shows further speciation apart in the form of mammalian-specific toxicity, likely stemming from divergent evolution from general toxicity. Currently, insecticidal-like forms of TC toxins that exhibit specificity towards mammalian cells are reported in *Y. pestis* and *Y. pseudotuberculosis*, with TC toxin in *Y. pseudotuberculosis* displaying reduced insecticidal activity and TC toxins in both *Yersinia* spp. exhibiting varying degrees of toxicity towards mammalian cells. In *Y. pestis*, TC toxin putative role in mammalian pathogenesis becomes much more pronounced as the possibility of TC toxin functioning as transmission factor was refuted. Other than the toxin playing an important part in phagocytosis resistance post-transmission, it exhibit qualities that suggests preferential upregulation in flea vectors or environments that stimulate colonization within flea vectors, although there appears to be a possibility of upregulation within host macrophages. This post-transmission resistance suggests if *Y. pestis* TC toxins can be proven to be T3SS independent, it is probable TC toxins might provide post-transmission resistance to phagocytosis during the initial stage of infection and upregulation of other T3SS-dependent virulence factors. However, research outside of TC toxins' renowned insecticidal activity is exceedingly limited, especially in bacteria other than *P. luminescens*, and areas for further research

is vast. If insecticidal-like TC toxins novel function within mammalian host can be conclusively determined, there can be preclinical prevention measures implications of Plague regarding the putative post-transmission role of TC toxin within *Y. pestis*, and perhaps in other microbes with possible insecticidal-like TC toxin homologs as well such as *M. morganii*. Regardless, all areas from stoichiometry to applications are in need of further research.

Acknowledgement

The authors thank Dr. Pravech Ajawatanawong for reviewing and providing feedback on this manuscript.

Conflict of interest

The authors declare no conflict of interest.

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