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THE ROLE OF TYPE IV SECRETION SYSTEM IN BRUCELLA VIRULENCE FACTOR: A REVIEW

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ABSTRACT

Brucellosis is a bacterial disease of domestic and wild animals caused by the genus Brucella which has great public health importance globally. In general, Brucella species do not express toxins or virulence factors that cause direct damage to the host. Instead, this pathogen's strategy is to persist long enough in the infected host until transmission can occur, which in the natural hosts is usually through abortion, sexual con-tact, or shedding of bacteria in milk. One important Brucella virulence factor for intracellular survival and persistence in the host is the type IV secretion system (T4SS).The type IV secretion system (T4SS) is one of numerous secretion systems used by microbes to transfer macromolecules through the cell membrane, such as proteins and DNA. It's the most versatile secretion system, transporting monomeric proteins, multi-subunit protein poisons, and nucleoprotein complexes, and it's found in Gram-positive and Gram-negative bacteria, as well as some archaea. The type IV secretion system is a major Brucella virulence factor for intracellular survival and host persistence. This review will go through the present state of knowledge on the Brucella type IV secretion system, including its architecture and regulation, as well as the newly discovered effector substrates that this system delivers into host cells.

Key words: Type IV secretion system, Virulence factor,Brucella

1. INTRODUCTION

Brucellosis is an economically important disease in production animals worldwide [1] and is one of the most common zoonotic infections. *Brucella* species have evolved to avoid the host's immune system and infection is usually characterized by long-term persistence of the bacteria. One important *Brucella* virulence factor for intracellular survival and persistence in the host is the type IV secretion system.

Type IV secretion systems (TFSS) are a newly identified family of multiprotein complexes that secrete macromolecules. [2]. T4SS is encoded by the virBoperon in Brucella, which is made up of 12 genes (virB1–12) on chromosome II. The promoter upstream of virB1 controls transcription of the virB operon.[3], [4]. The virBoperon was discovered in B. suis for the first time.Following that, it was discovered to be substantially conserved in all Brucella spp. for which genomic sequences were available, indicating that this operon may play a significant role. [3]. For a long time, it was assumed that Brucella spp. did not have the virulence factors found in other bacteria. [5].However, in recent years, various virulence factors that are essential for infection, including lipopolysaccharide [6], β-cyclic glucan[7], BvrS/BvrR[8] and outer membrane proteins (Omps) [9] have been identified. In Brucella, VirB T4SS is another major virulence factor that mediates intracellular survival and controls the host immunological response to infection .As a result, the role of the type IV secretion system in Brucella virulence factor will be discussed in depth in this review, including current knowledge of architecture and regulation, as well as the newly found effector substrates that this system delivers into host cells.

2. Regulation of the Brucella T4SS

When Brucella enters the host cell, it is exposed to novel environmental circumstances that encourage the production of VirB proteins.T4SS is only required by Brucella during specific and relatively short periods of intracellular infection, hence its expression must be tightly regulated. This period begins several hours after the bacterium is taken into a host cell and ends after phago-lysosome destruction has been avoided and a replication-ready vacuole in the ER has been created. After uptake by host cells, acidification of the Brucella-containing vacuole induces T4SS expression [10] .This occurs after the phagosome transiently fuses with early and late endosomes and lysosomes[11] . Nutrient deprivation could possibly be a signal for virBgene expression. After switching the bacteria from a rich medium to a minimal media at a low pH, virBgene expression can be strongly induced in culture [12]. Around 5 hours after infection of host cells, virBgene expression reaches its peak. [13] .

2.1 Architecture of the Brucella T4SS

The BrucellaT4SS is a complex of 12 proteins that assembles in the envelope of the bacterium. Once assembled, this mechanism can transport effector proteins, from Brucella into infected host cells. The virB operon, which includes genes virB1 through virB12 and is regulated by a major promoter upstream of virB1, encodes the BrucellaT4SS. (Figure1). The majority of VirB proteins are similar to T4SS components seen in other bacteria, such as Agrobacterium tumefaciens and Bordetella pertussis [3]. Brucella strains lacking a functional T4SS are highly attenuated in macrophages and mice and in the natural host, the goat [3], [4], [14]–[16] .
Insertions in virBgenes or genes affecting virB expression have repeatedly been discovered in transposon mutagenesis screens aimed to uncover Brucella virulence factors [17]–[21] This highlights the significance of the T4SS in Brucella pathogenicity.However, not all VirB proteins are required for the T4SS to function properly. By eliminating each virB gene in B. abortus one at a time, it was discovered that virB1, virB7, and virB12 are not required for B. abortus persistence in mice. [14], [16], [22] . In Brucella or other bacteria that possess a T4SS, VirB1, VirB7, and VirB12 are not known to be part of the T4SS core translocation apparatus or the pilus (figure 1).As a result, these proteins may assist or increase T4SS function but are not required. VirB1, for instance, is a lytictransglycosylase that destroys peptidoglycan to make room for the T4SS to form. [23] .However this function could be redundant as the Brucellagenome encodes other similar enzymes[14] .

The core structure of a T4SS was shown to be composed of the proteins VirB8, VirB9 and VirB10 [24], [25]. This appears to be also the case in Brucella as these three proteins interact with each other[26]. Other proteins, such as VirB6 and VirB7, may aid in the development of the core complex by stabilizing it. [27], [28] . T4SS function is also dependent on the ATPases VirB4 and VirB11, which provide energy for T4SS assembly and transport of effector proteins. [29], [30]. The major component VirB2 and the minor component VirB5 make up the pilus. [31] and assembly of the pilus is mediated by VirB3[32] .The pilus is thought to attach to the host cell surface and to createa pore, through which effectors are trans located [33] .

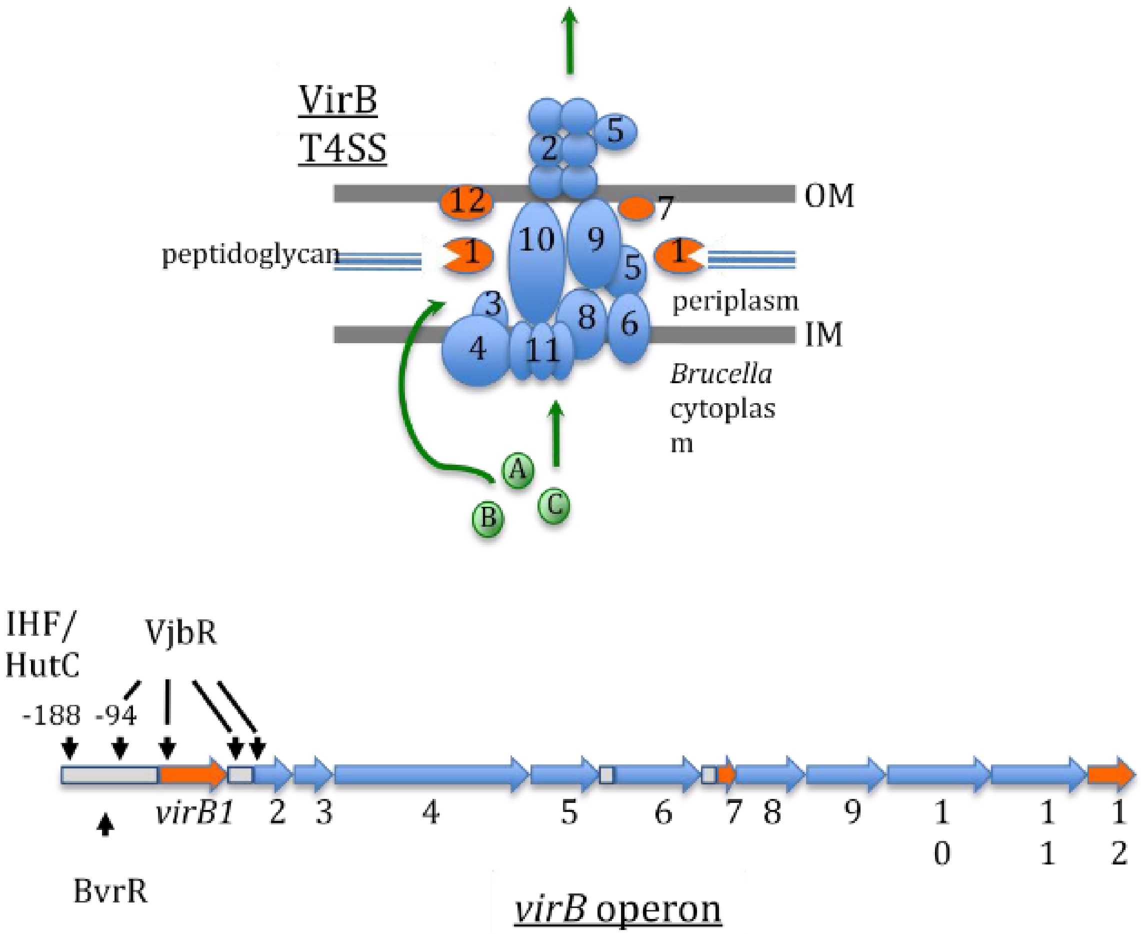

Figure 1.Schematic representation of the virB operon and the T4SS it encodes. In vitro in macrophages or in vivo in mice, the genes listed in blue are definitely essential for Brucella virulence. In mice, the genes highlighted in orange were determined to be non-essential for virulence. Direct regulators of the virB operon are also displayed, along with their (approximate) binding areas.Grey indicates putative promoters and intergenic areas containing putative promoters. Substrate proteins are shown in green and green arrows show putative translocation pathways.OM, outer membrane, IM, inner membrane.

2.3 Function of Brucella T4SS during infection

All T4SS virB genes are preserved in all Brucella species sequenced. Demonstrating that this system is vital for Brucella. The significance of the T4SS for Brucella virulence has been demonstrated experimentally, largely in vitro in macrophages and in vivo in a mouse infection model. [3], [4], [15], [18], [19]. Brucella wild-type bacteria can persist and reproduce in both models, however T4SS mutants are slowly eliminated.In vivo, B. melitensis and B. abortus wild-type bacteria induce innate immune responses in mice at later stages of infection, whereas T4SS mutants do not. [34]. This verified prior findings on Brucella's stealthy character during infection of their hosts, but it also indicated that later during infection, the T4SS serves as a signal that is recognized by the innate immune system, either directly or indirectly. (for review see[35] Brucella may actively translocate a molecule into host cells via the T4SS that activates the innate immune system with the purpose of polarizing the immune response to Th1 by increasing interferon gamma production or causing granulomas to develop. [35], [36].

It's also plausible that a variation in intracellular trafficking and growth causes the difference in immune activation between Brucell awild-type and T4SS-deficient strains. The BrucellaT4SS is essential for the maturation of the Brucella phagosome into an ER-derived compartment, according to experiments utilizing cultivated cells [11], [37], [38]. Despite the fact that Brucella phagosomes transiently fuse with early and late endosomes and lysosomes, a subset of intracellular Brucella with a T4SS is able to exclude endosomal and lysosomal markers from their phagosomes and avoid breakdown in phagolysomes. [11]. Brucella phagosomes, on the other hand, acquire ER markers such calreticulin. This process of excluding endosomal and lysosomal markers and acquiring ER markers is completed approximately 12 to 24 hours after Brucella infection of a host cell and requires the T4SS and presumably its translocated effectors. Brucella then starts to multiply to high numbers inside host cells, while Brucella virB mutants never reach the ER derived vacuole and are killed in phagolysosomes[11], [37], [38].

2.4 EFFECTORS

The genes encoding Brucella effectors identified to date are scattered across the two Brucella chromosomes (Figure2). In Bartonella species, the virBgenes are located together with the genes encoding the effector substrates[39] .Since a similar situation does not exist in Brucella, identifica-tion of Brucella effectors has proven to be a challenging task.

The first substrates of the BrucellaT4SS identified were VceA and VceC [40].These effectors were found in a screen for Brucella promoters that were activated by VjbR in the heterologous host E. coli. Although screening for effector genes among virBco-regulated genes is a great method of narrowing down potential effector candidates, many candidates could also have been missed. For example regulation of effectors may be under the control of a different regulator (such as directly by BvrR) or regulators down-stream of VjbR. Also it could be hypothesized that activation of some effectors is not connected to activation of the virBgenes. Brucella may already contain a ready for translocation‟ pool of effectors before entry into host cells, as has been shown for Legionella pneumophila[41] .These effectors could be required early during infection of the host cell and expressed constitutively. Since nutrients in the early Brucella phagosome are limited, hav-ing a ready pool of effectors would save resources for Brucella for other func-tions.

Recently, a different strategy to identify Brucella effectors was utilized by screening all proteins of unknown function for eukaryotic-like domains or do-mains known to be involved in protein-protein interactions [42]. This strategy has proven to be successful in identifying T4SS effectors of other intracellular pathogens, such as L. pneumophilaand Coxiella burnetii [43], [44]. Using this strategy, 6 proteins were identified that were translocated into mouse ma-crophages by B. abortus. Translocation into cells of 4 of these Brucellaputative effector proteins (BPE123, BPE005, BPE275 and BPE043) was dependent on the VirB T4SS (table 1).

Recently another protein was found that was translo-cated in a T4SS-dependent manner into macrophages during infection with B. abortus. This protein, named RicA, was found in a screen for Brucella proteins interacting with human proteins predicted to be associated with phagosomes. RicA was demonstrated to interact with Rab2, a GTPase involved in trafficking [45]. Rab2 has been shown before to localize to the Brucella phagosome. Furthermore, it was determined that Rab2 is important for intracellular replication of B. abortus [46]. In line with this, RicA, which preferentially binds to GDP bound Rab2, is involved in recruit-ing this GTPase to the Brucella phagosome [45] .

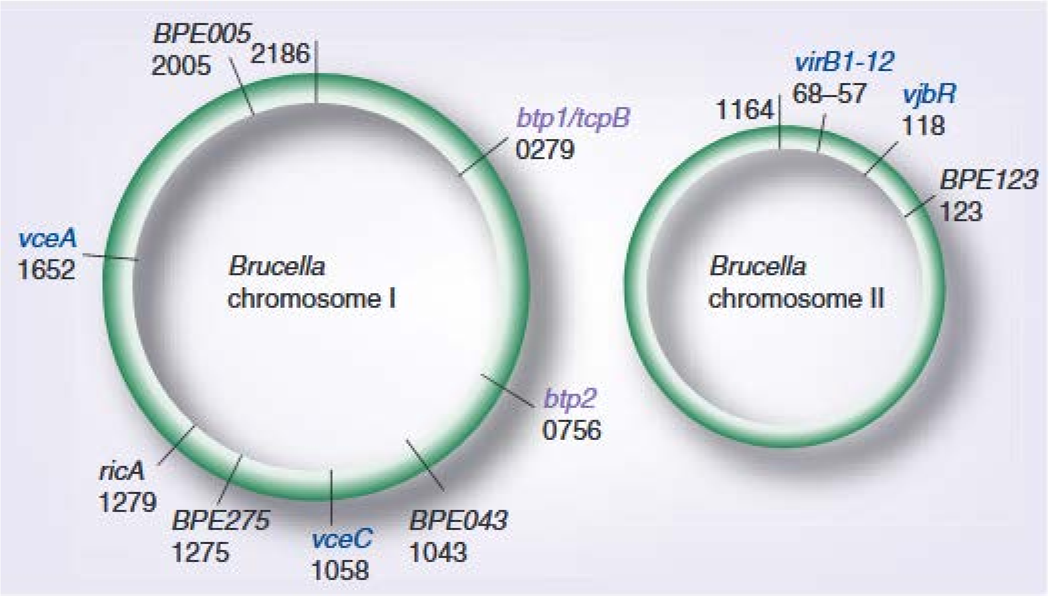

Figure 2. The two chromosomes of Brucella species showing the positions of effector genes, virBgenes and vjbR in the chromosomes. Effectors, shown in black and blue, have been shown to be translocated by the type IV secretion system into host cells. Those in purple have not been shown to be translocated to date. Genes shown in blue have been shown to be regulated by vjbR.

2.5 EFFECTORS

To date, 15 T4SS effectors have been identified in Brucella.The phenotypes presented by T4SS-deficient mutants and studies on some well-known T4SS effectors from other bacteria suggest that, in Brucella, T4SS effectors might function in the following molecular events associated with infection: (1) excluding markers of late endosome or lysosome, (2) acquiring ER markers, (3) interacting with secretory pathways, (4) acquiring markers for autophagosomes, (5) resisting the harsh intracellular environment, and (6) regulating the activation of vital immune pathways. However, their exact mechanisms of action have not been clarified. Every event may be targeted by one or more effectors, and an effector may act on one or more processes. In addition, the effectors may exhibit overlapping functions, thereby complicating the elucidation of their individual functions.

Although T4SS was identified in Brucella spp. a proximately 15 years ago, the effectors of T4SS remained uncharacterized until recently. The first two effectors, VirB-co-regulated effector (Vec) A and VecC, were identified while screening for genes whose transcription was co-re gulated by the virB operon regulator, VjbR [40]. Screening of th e i nteractions between human proteins and predicted Brucella proteins using a yeast two hybrid (Y2H) system led to the identification of the Rab2 interacting conserved protein A (RicA)-Rab2 interaction pair. Subsequently, RicA was identified as a T4SS-dependent effector [45] .Genome wide bioinformatics screening for putative T4SS effector proteins using distinct filtering criteria led to the identification of nine T4SS-dependent effector proteins [42], [47].

Bacterial toll-Interleukin receptor (TIR) domain-containing proteins are thought to be involved in the virulence of Brucella; screening for TIR-containing proteins in Brucella led to the identification of two proteins, Brucella TIR protein (Btp) A and BtpB, as effectors that are translocated by T4SS into host cells [48]. Recently, a secretory protein, secreted effector protein A (SepA), which is encoded within a horizontally transmitted region, was confirmed as a novel T4SS substrate [49].Together, these 15 effectors constitute the repertoire of T4SS substrates in Brucella spp. identified to date.

A candidate protein may be identified as a T4SS effector protein if it fulfills two criteria: the protein must be secreted into host cells and the secretion must be through the T4SS machinery. The former may be validated by TEM-1 lactamase or calmodulin-dependent adenylate cyclase (CyaA) assays and the latter, by constructing a T4SS-deficient mutant. All strategies used to identify effectors in Brucella are confined by the limited number of potential proteins; similar problems were encountered during the identification of effectors in other bacteria as well.By extending the screening of potential targets to the whole genome, the Dot/Icm T4SS machinery of Legionella pneumophila and Coxiella burnetii were identified to secrete approximately 300 and 100 proteins, respectively[44]. Similarly, extending the screening method described above to the whole Brucella genome may lead to the identification of many more effectors.

3. CONCLUSION

Brucella relies on multiple virulence factors, including the VirB T4SS, to induce the chronic illness brucellosis in people and animals. More than 10 years ago, the T4SS was discovered as a significant Brucella virulence component. Other significant human bacterial pathogens, such as Helicobacter pylori and Legionella pneumophila, have been identified to use a similar T4SS to translocate effector proteins into host cells, thus it was assumed that Brucella would do the same. Although a direct role for the T4SS in intracellular phagosome trafficking cannot be ruled out, the Brucella T4SS has been implicated in the translocation of a variety of potential effectors in infected host cells. Now that several substrates of the Brucella T4SS have been identified, the next step will be to determine the role of these putative effectors in intracellular survival of Brucella. Effector function may theoretically be divided into many categories depending on the host cell pathways that Brucella is known to disrupt, such as intracellular trafficking of the Brucella phagosome, host immune response manipulation, and apoptosis inhibition.

Some Brucella effectors may have a single function in the host cell, whereas others may have many functions. RicA probably interferes with Brucella phagosome trafficking by recruiting the trafficking GTPase Rab2 to Brucella phagosomes, which is one of the probable effec-tors reported thus far[45]. Although Btp1/TcpB has not yet been proven to be a T4SS substrate, the putative effector's known role is inflammation inhibition. Future study is likely to uncover new, and potentially surprising, host cell pathways that are manipulated by Brucella effectors, as well as explicate their role in Brucella infection.

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