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Review

Progresses on bacterial secretomes enlighten research on *Mycoplasma* secretome

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ABSTRACT

Bacterial secretome is a comprehensive catalog of bacterial proteins that are released or secreted outside the cells. They offer a number of factors that possess several significant roles in virulence as well as cell to cell communication and hence play a core role in bacterial pathogenesis. Sometimes these proteins are bounded with membranes giving them the shape of vesicles called extracellular vesicles (EVs) or outer membrane vesicles (OMVs). Bacteria secrete these proteins via Sec and Tat pathways into the periplasm. Secreted proteins have found to be important as diagnostic markers as well as antigenic factors for the development of an effective candidate vaccine. Recently, the research in the field of secretomics is growing up and getting more interesting due to their direct involvement in the pathogenesis of the microorganisms leading to the infection. Many pathogenic bacteria have been studied for their secretome and the results illustrated novel antigens. This review highlights the secretome studies of different pathogenic bacteria in humans and animals, general secretion mechanisms, different approaches and challenges in the secretome of *Mycoplasma* sp.

1. Introduction

A catalog of several pathways involved in the transport of proteins from the cytoplasm to cellular membrane and ultimately out of the cells, together with the proteins being transported, are defined as secretome. Proteins after synthesizing in ribosomes are transferred to cell walls where they have two ways: one way by which the proteins are cleaved into mature part and enter into extracellular space known as secreted proteins, while the other way is to be impregnated in the cell wall without entering the extracellular space known as surface-exposed proteins [1]. Sometimes, secreted proteins are overlapped with specialized membranes by forming vesicles, and hence, they are also called as extracellular vesicles (EVs) or outer membrane vesicles (OMVs) [2,3].

It is obvious that bacterial secretome is involved in the pathogenesis of numerous diseases in humans and animals. Studies about the secreted proteins of bacteria generate the chances to look into the new mechanisms involved in the pathogenesis of responsible pathogens. Discovery of novel biomarkers aids in the early diagnosis of several diseases with unknown or poorly understood pathogenesis. Till now, secretomes of significantly important bacterial pathogens i.e. *E. coli*, *Clostridium* sp., *Mycobacterium* sp., *Salmonella* sp., *Bacillus* sp., *Corynebacterium* [4–10] and few species of *Mycoplasma* have been studied for the identification of novel diagnostic markers and vaccine candidates. Because of fruitful outcomes, many scientists are looking forward to find something novel that can be the final solution to control some important bacterial diseases.

Mycoplasma sp cause various infections in human and animals but

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their secretomes are mostly untouched yet. Secretomes on *M. capricolum subsp. capricolum*, *M. acholeplasma laidlawii* PG8, *M. flocularae* and *M. pneumoniae*, *M. synoviae* have revealed some mysteries that contribute to vaccine development [11–15]. Although secretome studies are up to the mark but still there is a lacuna that indicates some obstacles to make it highly comprehensible. For instance, studying *Mycoplasma* secretome offers many challenges i.e. culture conditions, serum-free media, dead cell contamination specifically and prediction analysis generally that hinder to potentially uncover the secrets of secretome. The goal of this review is to get a breakthrough in *Mycoplasma* secretome by borrowing knowledge and techniques from bacterial secretomes with the summary of relevant research updates.

2. Classification of secreted proteins

2.1. Classical and non classical secreted proteins

Bacteria secrete the proteins by various pathways and each pathway is ornamented with some components that mediate the secretion system. Among these, two conserved systems are Sec and Tat pathways that transport unfolded and folded proteins respectively (explained in the next section). Proteins secreted via these classical pathways are known to be classical secreted proteins [16]. However many proteins that are found in the supernatant of many bacteria and lack signal peptides are known as non-classical secreted proteins [17]. There are intermingled concepts about the real media of secretion for these proteins. One school of thought suggests their secretion by some unknown secretion pathway [18,19], while other supports that it is the cell lysis that makes these proteins come into the extracellular milieu as majority of these proteins are cytoplasmic [20,21]. Many secretomic studies have reported non-classical proteins as a major part of secretome from specific bacteria [22–25].

2.2. Extracellular vesicles (EVs) or outer membrane vesicle (OMVs)

Extracellular vesicles generally known as outer membrane vesicles are bilayered nanosized entities harboring various functionally important components such as proteins, lipids, genetic material and metabolites [26]. Bacteria shed these vesicles that possess important biological functions delivered to the hosts to update bacterial virulence. Initially, it was assumed that EVs are only released from Gram negative bacteria while it was ignored in Gram-positive bacteria due to thick cell wall, but later it was found that Gram-positive bacteria also shed EVs [27]. However, the size of vesicles from Gram-positive bacteria was less (20–100 nm) as compared to the size of vesicles secreted from Gram-negative bacteria (20–200 nm). Generally, OMVs are produced due to blebbing of outer membrane in bacteria (Gram positive and Gram negative) and are composed of the outer layer of lipopolysaccharides and inner layer of phospholipids. Because of their origin from outer membrane, OMVs harbor a large variety of surface-associated proteins [28,29]. Basic mechanism involved in the generation of OMVs is disordered crosslinking between peptidoglycan and outer membrane. Shedding of OMVs due to cell lysis is also an acceptable phenomenon [30] and is another chapter of discussion. Here we are mentioning the causes other than cell lysis. Failure in an ordered and systemized array of crosslinking leads to the empty spaces that act as windows for OMVs. Defects in crosslinking of some mutants show overproduction of vesicles so it is important to take care for not affecting the genes involved in crosslinking while developing the mutant library. Growth conditions play a key role in vesiculization; high temperature favors vesicles generation. Other factors involved are flagellar movement in sheath that allows OMVs to come out and help in virulence of bacteria, signal molecules mediating their own transportation across the cell membrane [31], accumulation of misfolded proteins [32,33], phospholipid accumulation [34] and antibiotics [35] that weaken the cell membrane (Fig. 1). These have the ability to deliver virulent factors from bacterial cell surface to the host cells. Vesicles derived from *Streptococcus pneumoniae* are enriched with factors that contribute to disease progression. Results showed that EVs are highly immunogenic and are protective as immunizing agents [36]. EVs from *Mycoplasma synoviae* revealed many secreted proteins that were involved in host-pathogen interaction such as phosphodiesterase and kinase proteins which were found to be immunogenic and assumed to play critical roles during infection [12]. OMVs secreted from *E. coli* protect the pathogen and other bacterial communities i.e. *P. aeruginosa* from membrane active antibiotics i.e. mellitin and colistin [6]. Vesicles from *Salmonella* translocate virulence factors to the infected cells where they help in establishing the optimum environment for bacterial proliferation. Among these, PagK homologous proteins and lysosome-associated membrane proteins (LAMPs) were found to be secreted from OMVs that owe critical roles in bacterial virulence [4]. *Campylobacter jejuni* also releases vesicular proteins such as N-glycoproteins which have important biological functions by acting as reliable sources of protein delivery to the host cells [37]. In the same way, many pathogens such as *Vibrio cholera*, *Haemophilus influenza*, *Strenotophomonas maltophilia* and various *Mycoplasma* species release vesicles that play a significant role in bacterial virulence, survival, biofilm production, delivery, immunomodulation and other biological functions crucial for bacterial pathogenesis [13,26,38–40]. By using the advantages of EVs in small size, safety and high biocompatibility, EVs mimetic might be generated for development of novel vaccines and drug delivery tools [41].

3. Signal peptide cleavage and release

Peptides located at N-terminal of secreted proteins that play a crucial role in protein secretion pathway and target location are referred as signal peptides (SPs). Generally, a signal peptide consists of 3 regions; N region that acts as a positively charged domain, H region that is a hydrophobic core and C region is a cleavage site [43]. At the end stages of protein translocation, signal peptides are cleaved by peptidases present in the periplasm or at the outer surface of the cell membrane [44]. Proteins secreted by Sec and Tat pathways exhibit signal peptides that act as zip code for their translocation while secreted mechanisms involved for the transport of non-classical proteins is still an unrevealed mystery. Most prominent functions of signal peptides in protein translocation include inhibition of early folding of newly synthesized proteins, interaction with particular recognition proteins, interaction with proteins playing roles in transmembrane channels and ultimate interaction with signal peptidases [45]. Tat pathway study in *E. coli* revealed that TatBC complex directs the signal peptide into the membrane that penetrates halfway in lipid bilayer and the hairpin of this signal peptide is associated with the groove across the TatC. This hairpin is responsible for the translocation of mature domain across the membrane [46].

Most commonly used but highly conserved systems to transport the proteins across the bacterial cell membrane are the Sec secretion and twin arginine translocation (Tat) pathways. Proteins that are assisted by these mechanisms for transportation reside in periplasm or inner membrane of the cell. In Gram-negative bacteria several other mechanisms are involved to transport the proteins from cellular compartments to the outer cell milieu. Both systems mentioned above possess common elements involved in protein transportation but have primarily different mechanisms [16]. Secreted proteins are known to be transported by co-translational (involving ribosome) or by post-translational pathways that occur in eukaryotes and prokaryotes respectively [47].

3.1. The Sec secretion system

This pathway is responsible to transport unfolded forms of secreted proteins. It consists of three fundamental parts: protein targeting element, motor protein and a protein core complex known as SecYEG translocase [48]. Several pathogenic bacteria use Sec system for the

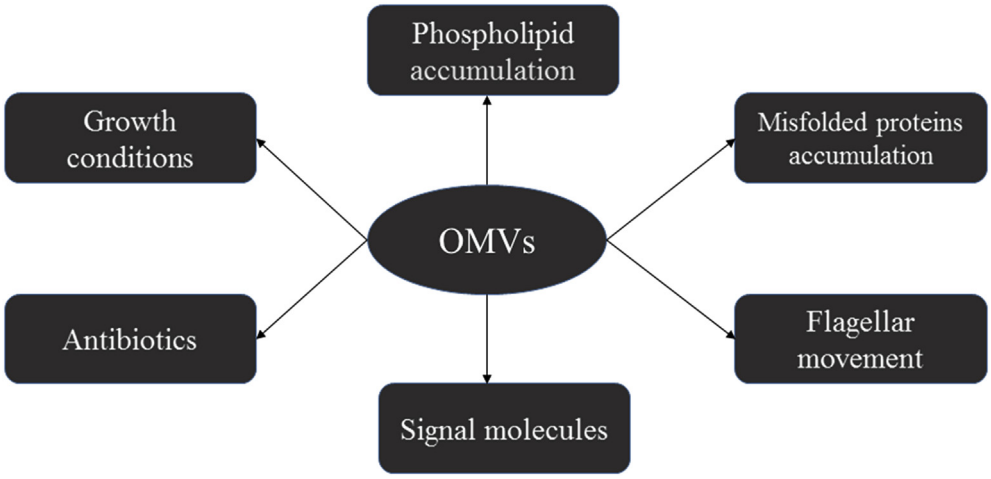

Fig. 1. Shedding of OMVs. Factors other than cell lysis are involved in shedding of OMVs [42].

translocation of virulent factors while some pathogens e.g. *Listeria monocytogenes* and *Staphylococcus aureus* produce a bunch of proteins that aid in the secretion of other major secreted proteins hence called as Sec accessory proteins and process is called Sec accessory system [49].

Specific signal sequences residing at N-terminus of secreted proteins play basic role for transportation in Sec system. On the basis of these signal sequences, Sec system is divided into two different pathways of protein transport. First, the pathway in which proteins are transported in periplasm or out of the cells has Sec-B specific signal sequence hence called SecB pathway (Fig. 2) [48]. SecB acts as a chaperon and targets the protein soon after translation from ribosome and hampers it from folding. The protein substrate is then directed to the motor protein SecA (a dimer) that has the function to navigate the protein towards SecYEG complex as well as consumes ATP to provide the energy for protein transportation across the channel suggesting the role of SecA as an ATPase [16,50]. SecA delivers the post-translational protein to the SecYEG complex linked with membrane where SecDF supports it and assist to drag the protein into the periplasm where it resides or finally becomes extracellular [16,51].

Second pathway is followed by the proteins that linger in the inner membrane and have signal recognition particle (SRP)-specific signal sequence hence known as SRP pathway (Fig. 3). It is found that affinity of SRP for translating ribosome is more as compared to the motor protein SecA [52]. After binding to the ribosomal protein (Ribosome-protein complex), SRP binds the FtsY protein that directs the complex to the SecYEG channel where it escapes and stays within the membrane [16,48,53].

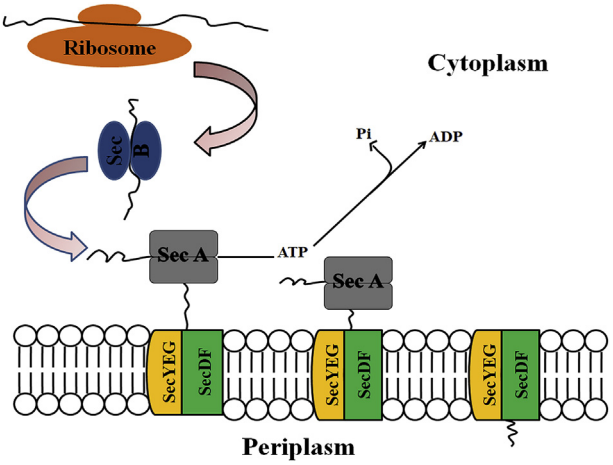

Fig. 2. SecB pathway of Protein secretion. SecB accepts the postranslated protein and directs it to the SecA that translocates the protein through SecYEG channel where SecDF supports to drag the protein towards periplasm [16].

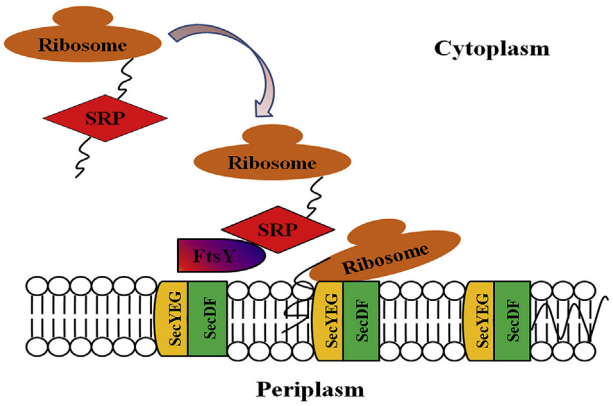

Fig. 3. SRP pathway of protein secretion. After binding to the ribosomal protein (Ribosome-protein complex), SRP binds the FtsY protein that directs the complex to the SecYEG channel where it esapes and stays within the membrane [16,48].

3.2. Tat secretion system

The most distinct feature of this secretion pathway is the transportation of folded proteins (Fig. 4) and works in bacteria, archaea and thylakoid membranes. In Gram-negative bacteria, whole pathway is based on TatABC substrate-binding complex and TatA complex. However, Gram-positive bacteria own a simpler mechanism having a bifunctional TatA instead of TatB element [54]. This mechanism is best known in *E. coli* where TatA and TatB have almost 25% similarity having identical secondary structures though TatB is more in length because of a stretched domain at C-terminus [55]. The protein secreted through tat pathway harbors a pair of twin arginine in S-R-R motif (RR precursor) at the N-terminus which acts as a signal sequence to attach with TatA and TatB components [56]. Many substrates from Tat pathway are secreted proteins in nature and redox in functions that play a role in anaerobic respiration. Other functions involved are biogenesis and re-modeling of cellular envelope and their absence causes impaired cell membrane and permeability. Most important function of tat secretion system is the transportation of virulent factors from pathogen to host cell [53]. It has been well known that TatC is the prime component to promote the attachment of RR precursor with substrate binding complex. Cross-linking has been found to be in between N-terminus of signal sequence and first cytosolic loop of TatC [57].

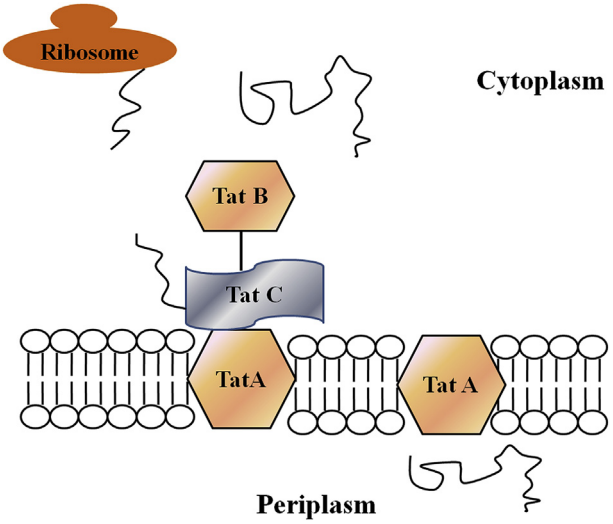

Fig. 4. Tat secretion system. Protein from ribosome attaches with TatB which fuses it with TatC. Protein is then directed towards periplasm via TatA chanel [16,54].

4. Involvement of bacterial secreted proteins in pathogenesis

Bacteria release extracellular vesicles that are enriched with proteins, lipoproteins, glycolipids, phospholipids and genetic materials. These EVs transfer the virulence factors to the host cells and have been incriminated in bacterial pathogenesis resulting in several important diseases in humans and animals [58]. These vesicles do so by invading and destroying the host cells, escaping immune surveillance and or promoting the antibiotic resistance [59]. After their entry into the host cells, EVs induce various pathophysiological functions such as non-immunogenic and acute and chronic inflammatory responses. For example, EVs secreted by *Escherichia coli* are responsible for the induction of leukopenia, pro-inflammatory cytokines, hypotension and lung abnormalities in mice. In the same way, EVs released by *Pseudomonas aeruginosa* and *Klebsiella pneumonia* induced inflammatory responses in the lungs. *Staphylococcus aureus* is also known to secrete the vesicles that are involved in pulmonary inflammatory responses [41]. It is interesting to put into notice that almost every study related to bacterial secretome has brought something useful about the pathogenesis of concerned bacterial pathogens. This truth came into existence because secreted proteins are well known to be involved in immunomodulation, carry and deliver the virulent factors and toxins, drug delivery and aid in biofilm formation. Extracellular proteins of various pathogenic bacteria possess pathological and physiological functions such as inflammatory responses [60], septic shock [61], neutrophil infiltration [62], atopic dermatitis [63] and airway hypersensitivity [64]. Intracellular survival of bacterial pathogens is also facilitated by secreted proteins. *Campylobacter jejuni* secretes a protein CiaI that is *Compylobacter* invasion antigen involved in intracellular survival and expedites the existence of bacteria in the host epithelial cells. It was hypothesized that CiaI prevents *C. jejuni* to fuse with lysosomes and hence it gets risk free environment to invade the tissues [65]. Secreted proteins might be utilized as antimicrobial chemotherapy as shown in the secretome study of *Helicobacter pylori.* Secreted oxid reductases and HtrA were found to be the best targets for antimicrobial agents [66]. All these findings reveal the supreme role of secreted proteins in bacterial pathogenesis and diagnosis.

5. Progresses of research on bacterial secretome

5.1. E. coli secretome

Secretome study on Adherent-invasive *Escherichia coli* (AIEC) and Enterotoxigenic *E. coli* revealed many proteins that were involved in virulence. Novel vaccine candidates of both strains were identified and found that YghJ and surface adhesin antigen help pathogen to adhere to the intestinal cells and also serve for the transport of heat labile toxins in a befitting manner. These results support that YghJ is responsible for the colonization of pathogen and aids to exaggerate the degradation of host intestinal cells by deteriorating the intestinal mucin i.e. MUC2 and MUC3 [67]. Some pathogenic bacteria have also been investigated for their ability to secrete proteins concerned with specific secretion system. Among various secretion systems described above, type III secretion system is pivotal for the virulence and pathogenesis of Gram-negative bacteria [68]. So, secreted proteins might be the way left for the development of a proper vaccine candidate for such pathogen. By keeping this view, Enteropathogenic *E. coli* secreted proteins involved in type III secretion pathway were investigated and C_0814/NleJ and LifA were identified as innovative proteins translocated into host cells. LifA factor plays a pathogenic role as both an adhesin and a toxin [69].

5.2. Helicobacter pylori secretome

Helicobacter pylori revealed a set of secreted proteins that were predicted to be putative vaccine candidates against gastric disorders such as peptic ulcer and malignancy. Host-pathogen interaction analysis confirmed direct or indirect involvement of vacA, babA, sabA, fecA and omp16 in cell signaling mechanisms and explained that these secreted proteins can serve as potent vaccine candidates [70]. A lot of researches are ongoing to elaborate the secretome of different pathogenic bacteria and their outcome is much reliable in a case to find out some significant factors needed for development of novel vaccines as well as the diagnostic reagents.

5.3. Bordetella pertussis secretome

Bordetella pertussis secretome was studied using different types of media and it was found that eight secreted proteins harvested from Thalen-Ijssel (THIJS) media were specifically associated with virulence for example FhaL & FhaS as adhesins, BP1251 as a putative toxin and T3SS protein. However, none of the proteins harvested from Stainer-Scholte (SS) media were associated with virulence [71]. Culture conditions are very important in order to find proteins involved in pathogenesis and to develop a future vaccine. Therefore, choosing the type of media is a critical and basic step in order to study the secretome of bacterial cells extensively.

5.4. Campylobacter jejuni secretome

Campylobacter jejuni was studied for its OMVs and 151 proteins were identified including the factors associated with pathogenesis such as cytolethal distending toxins (CDT). These findings suggested that *C. jejuni* has a cytotoxic ability and can elicit the immune response in host cells [38].

5.5. Mannheimia hemolytica secretome

Two strains (89010807 N and Oklahoma) of *M. haemolytica* were subjected for secretome assay. Total number of proteins obtained from both strains were 923. Out of them, 283 proteins were predicted to be secreted in nature. Various prediction softwares such as PRED-TAT, SecretomeP 2.0, SignalP 4.1, Phobius and LipoP revealed 172 (60.7%), 184 (65.0%), 114 (40.2%), 151 (53.3%), and 138 (48.7%) secreted proteins respectively. In addition, the proteins secreted via non-classical

pathway were 95 (33.56%) [24].

5.6. Corynebacterium pseudotuberculosis secretome

Comparative study of exoproteome for the two strains of *Corynebacteriumm pseudotuberculosis* by gel-free system (TPP-LC/MSE) revealed 93 extracellular proteins by using SurfG+ as prediction tool for subcellular localization. Among these, 44 extracellular proteins were found to be common between the two strains (1002 and C231). About 70 out of 93 proteins were predicted to have signal peptides for extracellular transportation [72]. Differential study on exoproteome of these strains showed 17 differential proteins between these two strains. Moreover, the number of overexpressed proteins in 1002 and C231 were 9 and 8 respectively [7]. Later on, exoproteome of these strains was studied by a different approach (MALDI-TOF/TOF) identifing 11 novel extracellular proteins that might have some role in virulence of this pathogen [7]. Therefore, characterization of the novel secreted proteins and finding their role in the virulence of bacteria can provide strong evidences for understanding the pathogenesis of *C. pseudotuberculosis*.

5.7. Burkholderia cepacia secretome

Two-dimensional gel electrophoresis was carried out to elucidate the immunogenic proteins released in the secretome of *Burkholderia cepacia*, and it was concluded that 18 proteins were immunogenic and might be the source of potent vaccine or diagnostic markers against anomalies caused by *B. cepacia* [73]. Secretome is also helpful to detect the virulence level of bacterial strains based on the presence or absence of lethal factors within the host cells. Secretome study is getting more interesting by time because of its functional diversity and reliability as well as thought-provoking findings.

5.8. Listeria monocytogenes secretome

Virulence ability of four strains of *Listeria monocytogenes* was identified by the secretome analysis of these strains. It was concluded that the levels of internalin C (InIC) and listeriolysin O (LLO) that exist in secretome determines the expression of factors responsible for virulence [74]. Comparative study on the secretome of pathogenic strains or closely interrelated species may assist in the identification of shared secreted proteins that can lead to the development of a common vaccine or diagnostic markers of two or more different diseases.

5.9. Bacillus clausii secretome

Differential secretome among bacterial strains helps to indicate the virulent properties associated with specific strain such as *Bacillus clausii* probiotic strains [9]. Many pathogenic bacterial secretome has been probed and found crucial virulence or pathogenesis related factors that can be the source of diagnostic markers or candidate for effective vaccine development. Rather than the numbers of studies that have been conducted in this field, the investigation of secretome at molecular level remains in need. Whereas the identification of various secreted proteins and their pathogenic functions including cell interaction, adhesion, invasion, apoptosis and/or inflammatory response might add a great progress in this field. Such kind of studies may lead to the discovery of novel antigens that might be useful as diagnostic markers, vaccine and drug targets.

5.10. Staphylococcus aureus secretome

Secretome of methicillin-resistant *Staphylococcus aureus* clone was studied extensively and was found to possess 174 obvious proteins. Among these were proteins responsible for various functions such as protein synthesis (16.09%), virulence (13.79%), toxins (6.89%) and 17.24% with unknown functions (17.24%). Enterotoxins U and B were firstly identified in this study [75].

5.11. Streptococcus pneumoniae secretome

Proteomics study explored 42 secreted proteins of *S. pneumoniae* possessing signal peptides. According to the subcellular localization 21 and 4 proteins were localized in extracellular milieu and cell wall respectively. Gsp-781, PhtD, Sphtra, Eno, NagA and ZmpB were found to be potent immunogenic secreted proteins. Among these, Gsp-781 was presented as novel secreted protein of *S. pneumoniae* [76].

5.12. Streptococcus agalactiae secretome

Study on the secretome of *Streptococcus agalactiae* revealed secreted proteins and rIsp was shown to have the ability to urge significant antibody titer in tilapia and regarded as protective antigens as it produced convincing protection against *S. agalactiae*. Proteins secreted from *Streptococcus suis* serotype 9 were analyzed and revealed 13 secreted proteins. Majority of these were associated with metabolic functions, while five were found to be virulence linked factors such as o-acetylserine lyase, phosphoglycerate mutase, DNA nuclease, putative 5′-nucleotidase and peptidoglycan-binding LysM. This analysis of secretome provided significant assistance to understand the pathogenic mechanisms of the most prevalent serotype in pigs [77]. It might be thought that the kind of media can affect the number and the functional type of secreted proteins released by specific pathogenic bacteria.

5.13. Mycobacterium tuberculosis secretome

Recent study on secretome of *M. tuberculosis* revealed some important proteins that can be applied for the diagnosis of tuberculosis infection. Among the identified proteins, PE3 and PE4 showed excellent reactivity against 158 serum samples from TB patients. However, CFP-10 and ESAT-6 also showed high response while other identified antigens were Ag85 A, Ag85B, Ag85C and MycP1. These newly identified antigens may play a significant role for low cost diagnosis of MTb infections [78].

6. Progresses of research on *Mycoplasma* secretome

6.1. Recognized secreted proteins

Some mycoplasmas secretome has been studied in order to improve the diagnostic tools and vaccines [11,12,14,15].

An analytic study on *Mycoplasma hyopneumoniae* and *Mycoplasma flocculare* secretomes was performed and the results revealed existence of 7 common proteins among the two species [15]. Recently, EVs from different species/subspecies of *Mycoplasma* were isolated and compared for their shape and size as well as harboring virulence factors. It was illustrated that smallest EVs belong to *M. agalactiae* strain L14628 and *M. bovis* strain L15762 while largest vesicles were produced by *Mycoplasma mycoides sub sp mycoides Afadé*. Several virulent factors were also detected in 3 species of *Mycoplasma* (*M. mycoides* subsp *mycoides* Afadé, *M. agalactiae* 5632, *M. fermentans* PG18). Noticeable virulent factors were P37, OppA, LppB lipoproteins, Vpmas, MAG5040, Ef-Tu, Hsp70, glucose permease involved in oncogenic functions, apoptosis, immunomodulation, phase variation and adhesins, escape from neutrophil extracellular trap, elongation factor, cytokine secretion and marker for semi-quantification of EVs [79].

Proteomic profile of *M. bovis* has been recently studied for the purpose to find a diagnostic marker for early diagnosis, to develop a new vaccine with high potency, and to reveal the pathogenesis to control the disease (Table 1). It was demonstrated that MbovNase is a nuclease secreted from *M. bovis* that has the ability to enter the macrophages and induce apoptosis [80]. It is a clue research about the

Table 1
Antigenic proteins of *M. bovis*.

Proteins	Functions	Localization	Reference
p26	Adhesin	Surface	[84]
MilA	Lipase activity, immunogenic, development of iELISA	Membrane	[85]
α-enolase	Adhesion related factor	Surface	[86]
VpmaX	Adherence to EBL cells	Surface	[87]
pMB67	Surface divergence	Surface	[88]
p48	Development of Dc-ELISA, specific marker of infection	Membrane	[89,90]
p81	Diagnostic testing using multiplex-PCR	Membrane	[91]
p40	Adhesion, pseudogene		[92]
p68	Macrophage-activating lipoprotein (MALP)	Membrane	[93]
Mbov_0579	rMbovP579 based ELISA, novel antigen	Membrane	[81]
GAPDH	Potential factor for the development of potent vaccine	Cytoplasmic	[39]
VSPs	Reduction in adherence of *M. bovis* to the bovine bronchial epithelial (BBE) cells	Membrane	[94]
p27	Adhesion to EBL cells mediated by fibronectin	Surface	[95]
HSP60	Immunogenic in natural infection	Surface	[96]

secreted proteins of *M. bovis* that illustrate how an extensive study regarding its secretome can reveal various important factors for virulence and pathogenesis of this pathogen. Before this, a study on the proteomic analysis was conducted and the immunogenic protein MbovP579 was predicted to be secreted in nature [81]. Another study predicted several putative secreted proteins in *M. bovis* that may be involved in various kinds of biological functions. Among these twelve proteins, eight proteins encoded by Mbov_0049, Mbov_0347, Mbov_0350, Mbov_0518, Mbvo_0579, Mbov_0679, Mbov_0729, Mbov_0797 have conserved domains which are conserved units in proteins associated with protein structure and functions [82]. Specific function allotted to the protein is dependent on the combination of domains [83]. In addition, at least 60 secreted proteins exist in *M. bovis* (data not shown). Since already predicted secreted proteins might be responsible for biological functions, further experimental identification of secretome involved in the pathogenesis of *M. bovis* would be encouraged.

6.2. Approaches to study Mycopalsma secretome

Output of secretome directly relies on the approach for the extraction and identification of secreted proteins. Various approaches are adopted to study the bacterial secretome at its level best to reduce the contamination and avoid the cell compartments due to lysis of the cells. For *Mycoplasma*, we face some specific challenges such as interruption by high concentration of serum in growth medium and leakage of intracellular compartments because of ease in cell lysis due to lack of cell wall. We will cover some important aspects that need to be practiced for studying *Mycoplasma* secretome.

6.2.1. Serum optimization/serum free media

Mycoplasma species are fastious and require media supplemented with serum for their efficient and optimum growth. Large number of proteins in the serum greatly interfere with secretome and subside less abundant secreted proteins. It is important to optimize the culture conditions with minimum amount of serum that on one hand won't affect the bacterial growth and on other hand shouldn't be enough to hide the secreted proteins. Various studies on *Mycoplasma* secretome [15] include this approach that results into better quality outputs. Serum free medium has also been practiced [12] and was found an effective approach for acquiring bacterial secretome. Another useful approach is to prepare the growth media supplemented with usual amount of serum and then filtered with Amicon filter (Millipore Stirred Cell 8400) with a 10,000 kDa cut off limit to remove the protein. The filtered media is then inoculated with bacterial culture to explore secretome [11,97]. Modifying medium while minimizing the serum concentration may affect the cell growth so one should optimize the conditions and growth should be followed by colony forming unit (CFU) or optical density (OD_{600}) [15].

6.2.2. Extraction and in-silico analysis

Generally, EVs can be extracted in the supernatant of culture by centrifugation or ultracentrifugation [13,73]. Proteins are precipitated by adding Ammonium sulfate [23] or 10–12% Trichloroacetic acid (TCA) [98]. After that, obtained secretome can be proceeded in two ways either by gel separation method (2-dimensional gel electrophoresis and SDS-PAGE) or by performing Label-free analysis. Proteins can be identified by LC-MS/MS analysis (Fig. 5). Bioinformatics tools are very important to analyze the secretome secreted by pathogens. Moreover, the prediction of signal peptides and functions related to the pathogenesis helps to attain the secretome profile of organisms and future work strategy [99]. Fig. 5 elaborates the general pathway to analyze the secretome data of specific bacteria. There are various web servers that can be availed to predict several properties associated with the proteins of interest. These online servers can be utilized for the prediction of subcellular localization, membrane topology, signal peptides and others (Fig. 5).

6.2.3. Pulsed stable isotope labeling of amino acids in cell culture (pSILAC)

Although Mass spectrometry is often practiced for proteomics analysis but for secretomics, it is not reliable as it can't differentiate between serum proteins and newly synthesized secreted proteins. Secretome analysis of serum containing cultures is facilitated by pSILAC approach together with azidohomoalanine (AHA) labeling [101,102].

6.3. Challenges faced in secretome research of Mycopalsma species

Mycoplasma sp are very essential and reported to cause diseases equally in human and animals. Proteomics research on *Mycoplasma* brought new insights to understand the pathogenesis and find out diagnostics markers. In spite of all this, there is a lacuna for understanding pathogenesis and sorting candidate for development of vaccine. As already mentioned, secreted proteins are very important for the search of some vaccine candidates but there are some challenges that hamper the way for the complete and accurate study of *Mycoplasma* secretome.

6.3.1. Method limitation

As *Mycoplasma* sp lack cell wall and have exposed cell membranes that may break with simple stress allowing cytosolic components to come out in extracellular space. During secretome extraction, there are more chances for this phenomenon to happen that can affect the secretome accuracy. Many studies revealed higher proportion of cytosolic localization of proteins and several proteins lacking signal peptides indicating the cell lysis during secretome extraction [24,71,75,103,104]. However, lack of signal peptides may indicate the non-classical nature of secreted proteins [18]. Amount of DNA in the cell culture may specify the degree of lysis [22] but EVs also carry

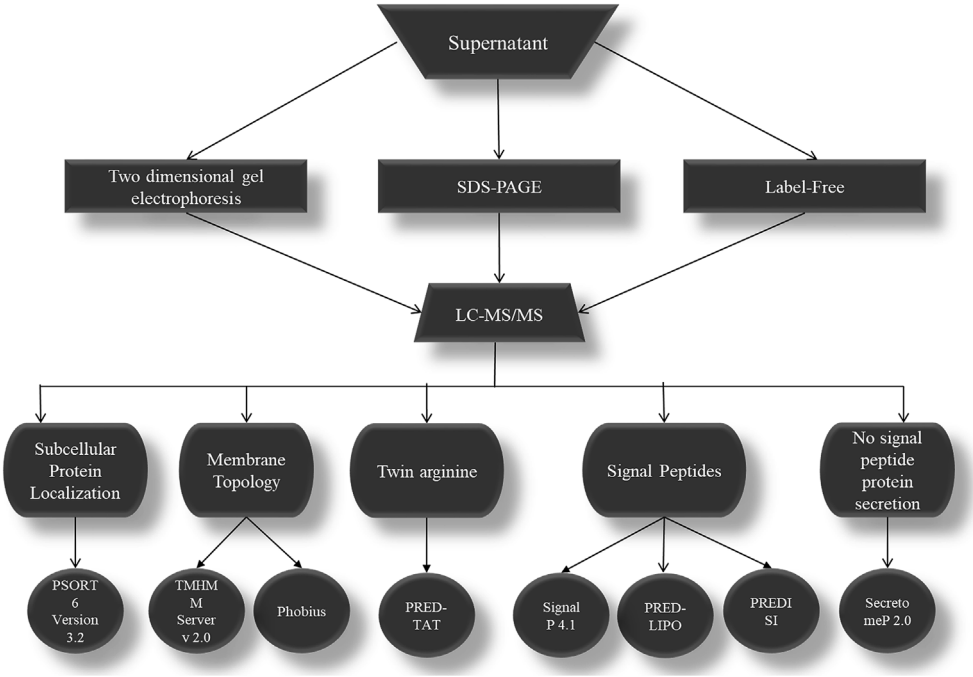

Fig. 5. Extraction, separation and identification of secretome. Extracted secretome is separated by SDS-PAGE or 2DE or is identified by Label-free analysis. LC-MS/MS identifies the proteins that can be analyzed by using various online prediction tools [100].

genetic material (DNA, RNA) [41] that again makes it complex. Other possibility to ensure whether the protein is secreted or released due to cytosolic leakage is the use of DAnTE program. Extracellualr and cytoplasmic fractions are compared using this program and very low correlation of these two fractions indicates the cytoplasmic proteins are not due to cell lysis. Measuring the mol% of extracellular sytoplasmic proteins during culture incubation period may also be helpful in this case. If the mol% change is not significant throughout the period or remains constant, it indicates less or no cell lysis respectively [76]. It is said that non classically secreted proteins have disordered structure as compared to cytoplasmic proteins [105]. Instead of all these measures, accurate secretome study is still a question mark and can be resolved by introducing some novel methods for extraction and identification of secreted proteins from *Mycoplasma* species.

6.3.2. Verification limitation

Another challenge in the study of secretome of *Mycoplasma* is the limitations for the confirmation of secreted proteins. Severalservers are available for the prediction of secreted proteins i.e.(classical or non-classical), subcellular localization (extracellular, cytosolic, cell membrane, cell wall, nucleus), signal peptides and other secreted pathways (Sec and Tat pathway) [100]. Experimental validation of all above aspects is not clear yet and even seems to be very complex. Discrimination between non-classical secreted proteins and cytoplasmic proteins is essential for identifying the accurate secretome. Current practices for the confirmation of secreted proteins is the blotting with whole cell and supernatant fractions but this method also has limitations as supernatant may have the lysed cell components giving false positive results. Another idea was to isolate all the fractions corresponding to the cells i.e. cell membrane fraction, extracellular fraction, cytosolic fraction and whole cell fraction and blotting with corresponding secreted protein antibodies. This expanded idea may give some satisfactory results for verification of secreted proteins. Confirmation of subcellular localization is another limitation for secretome assay. Although, localization confirmation is easy and has been practiced for some eukayotic secreted proteins [106,107] but it is very rare for bacterial secreted proteins. Secretome assay constitutes several secreted proteins that makes it difficult to experimentally verify the subcellular localization of each protein. Same is the case with signal peptides and pathway analysis of secreted proteins identified. However, consortium of secreted proteins can provide the understanding about the secreted system in bacteria. In *Mycoplasma*, secreted system is not well studied yet. However, secretome assay of *Mycoplasma bovis* highlighted some proteins (unpublished data) that can give clues to the Sec pathway (presence of SecA and SecYdf proteins). Future studies need to introduce various confirmatory tools and mechanisms to explore secreted pathways in *Mycoplasma* sp.

7. Prospect of future study in research on both bacterial and *Mycoplasma* secretome

Elaborative study on the secretome of some pathogenic bacteria can lead to a complete understanding of their poorly understood pathogenesis as well as the development of potential vaccine antigen. Most of the studies on the secretome of *Mycoplasma* sp are *in silico* approaches that are not enough to find out potential biomarkers. Functional analysis at the molecular and cellular levels such as adhesion, invasion, apoptosis and cell interactions can uncover the mysterious pathogenesis. Novel methodology is the need of the time that can reduce the cytoplasmic proteins in secretome avoiding cell lysis. In addition, the platform on genetic manipulation of *Mycoplasma* sp is critical to identify the individual functions of secreted proteins. It will be a great research step to tackle the mycoplasmosis in cattle and minimize the economic losses to a great extent.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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References

[1] A. Barinov, et al., Prediction of surface exposed proteins in Streptococcus pyogenes, with a potential application to other Gram-positive bacteria, Proteomics 9 (1) (2009) 61–73.
[2] E.Y. Lee, et al., Gram-positive bacteria produce membrane vesicles: proteomics-based characterization of Staphylococcus aureus-derived membrane vesicles, Proteomics 9 (24) (2009) 5425–5436.
[3] P. Mukherjee, S. Mani, Methodologies to decipher the cell secretome, Biochim. Biophys. Acta Protein Proteonomics 1834 (11) (2013) 2226–2232.
[4] H. Yoon, C. Ansong, J.N. Adkins, F. Heffron, Discovery of Salmonella virulence factors translocated via outer membrane vesicles to murine macrophages, Infect. Immun. 79 (6) (2011) 2182–2192.
[5] G.S. Niemann, et al., Discovery of novel secreted virulence factors from salmonella enterica serovar typhimurium by proteomic analysis of culture supernatants, Infect. Immun. 79 (1) (2011) 33–43.
[6] H.M. Kulkarni, R. Nagaraj, M.V. Jagannadham, Protective role of E. coli outer membrane vesicles against antibiotics, Microbiol. Res. 181 (2015) 1–7.
[7] W.M. Silva, et al., Differential exoproteome analysis of two corynebacterium pseudotuberculosis biovar ovis strains isolated from goat (1002) and Sheep (C231), Curr. Microbiol. 67 (4) (2013) 460–465.
[8] F. Cornejo-Granados, et al., Secretome prediction of two M. tuberculosis clinical isolates reveals their high antigenic density and potential drug targets, Front. Microbiol. 8 (FEB) (2017) 1–12.
[9] R. Lippolis, et al., Comparative secretome analysis of four isogenic *Bacillus clausii* probiotic strains, Proteome Sci. 11 (1) (2013) 28.
[10] A. Boetzkes, K.W. Felkel, J. Zeiser, N. Jochim, I. Just, A. Pich, Secretome analysis of clostridium difficile strains, Arch. Microbiol. 194 (8) (2012) 675–687.
[11] A. Voros, J. Delongchamp, M. Saleh, The secretome of mycoplasma capricolum subsp. capricolum in neutral and acidic media, J. Proteonomics Bioinf. 8 (7) (2015) 155–163.
[12] M.S. Rebollo Couto, C.S. Klein, D. Voss-Rech, H. Terenzi, Extracellular proteins of *Mycoplasma synoviae*, ISRN Vet. Sci. 2012 (2012) 1–6.
[13] V.M. Chernov, et al., Extracellular membrane vesicles secreted by mycoplasma Acholeplasma laidlawii PG8 are enriched in virulence proteins, J. Proteom. 110 (2014) 117–128.
[14] V.M. Chernov, et al., Extracellular vesicles derived from Acholeplasma laidlawii PG8, Sci. World J. 11 (2011) 1120–1130.
[15] J.A. Paes, K.R. Lorenzatto, S.N. de Moraes, H. Moura, J.R. Barr, H.B. Ferreira, Secretomes of Mycoplasma hyopneumoniae and Mycoplasma flocculare reveal differences associated to pathogenesis, J. Proteom. 154 (2017) 69–77.
[16] E.R. Green, J. Mecsas, Bacterial secretion systems: an overview, Virulence Mech. Bact. Pathog. Fifth Ed., 4 2016, pp. 215–239 1.
[17] G. Wang, Y. Xia, X. Song, L. Ai, Common non-classically secreted bacterial proteins with experimental evidence, Curr. Microbiol. 72 (1) (2016) 102–111.
[18] L. Zhao, J. Chen, J. Sun, D. Zhang, Multimer recognition and secretion by the non-classical secretion pathway in Bacillus subtilis, Sci. Rep. 7 (2017) 1–18.
[19] J. Chen, et al., A novel strategy for protein production using non-classical secretion pathway in Bacillus subtilis, Microb. Cell Factories 15 (1) (2016) 1–16.
[20] L. Oliveira, et al., Group B streptococcus GAPDH is released upon cell lysis, associates with bacterial surface, and induces apoptosis in murine macrophages, PloS One 7 (1) (2012).
[21] P. Ebner, J. Rinker, F. Götz, Excretion of cytoplasmic proteins in Staphylococcus is most likely not due to cell lysis, Curr. Genet. 62 (1) (2016) 19–23.
[22] L.D.W. Luu, S. Octavia, L. Zhong, M. Raftery, V. Sintchenko, R. Lan, Characterisation of the Bordetella pertussis secretome under different media, J. Proteom. 158 (2017) 43–51.
[23] W. Li, H.Q. Wang, R.Z. He, Y.W. Li, Y.L. Su, A.X. Li, Major surfome and secretome profile of Streptococcus agalactiae from Nile tilapia (Oreochromis niloticus): insight into vaccine development, Fish Shellfish Immunol. 55 (2016) 737–746.
[24] S. Ayalew, A.W. Confer, S.D. Hartson, P.J. Canaan, M. Payton, B. Couger, Proteomic and bioinformatic analyses of putative Mannheimia haemolytica secretome by liquid chromatography and tandem mass spectrometry, Vet. Microbiol. 203 (2017) 73–80.
[25] Y.T. Wen, J.S. Wang, S.H. Tsai, C.N. Chuan, J.J. Wu, P.C. Liao, Label-free proteomic analysis of environmental acidification-influenced Streptococcus pyogenes secretome reveals a novel acid-induced protein histidine triad protein A (HtpA) involved in necrotizing fasciitis, J. Proteom. 109 (2014) 90–103.
[26] T.N. Ellis, M.J. Kuehn, Virulence and immunomodulatory roles of bacterial outer membrane vesicles, Microbiol. Mol. Biol. Rev. 74 (1) (2010) 81–94.
[27] Ann L. Coker, 乳鼠心肌提取 HHS public access, Physiol. Behav. 176 (5) (2017) 139–148.
[28] A. Kulp, M.J. Kuehn, Biological functions and biogenesis of secreted bacterial outer membrane vesicles, Annu. Rev. Microbiol. (2012) 163–184.
[29] C. Schwechheimer, M.J. Kuehn, D.L. Rodriguez, NlpI-mediated modulation of outer membrane vesicle production through peptidoglycan dynamics in \emph {Escherichia coli}, Microbiologyopen 13 (10) (2017) 605–619.
[30] L. Turnbull, et al., Explosive cell lysis as a mechanism for the biogenesis of bacterial membrane vesicles and biofilms, Nat. Commun. 7 (2016).
[31] C. Florez, J.E. Raab, A.C. Cooke, J.W. Schertzer, Crossm membrane distribution of the, (2017), pp. 1–13.
[32] A.J. McBroom, A.P. Johnson, S. Vemulapalli, M.J. Kuehn, Outer membrane vesicle production by Escherichia coli is independent of membrane instability, J. Bacteriol. 188 (15) (2006) 5385–5392.
[33] A.J. McBroom, M.J. Kuehn, Release of outer membrane vesicles by Gram-negative bacteria is a novel envelope stress response, Mol. Microbiol. 63 (2) (2007) 545–558.
[34] S. Roier, et al., A novel mechanism for the biogenesis of outer membrane vesicles in Gram-negative bacteria, Nat. Commun. 7 (2016) 1–13.
[35] J.L. Kadurugamuwa, T.J. Beveridge, Virulence factors are released from, Microbiology 177 (14) (1995) 3998–4008.
[36] A. Olaya-Abril, et al., Characterization of protective extracellular membrane-derived vesicles produced by Streptococcus pneumoniae, J. Proteom. 106 (2014) 46–60.
[37] K.-S. Jang, M.J. Sweredoski, R.L.J. Graham, S. Hess, W.M. C Jr., Comprehensive proteomic profiling of outer membrane vesicles from Campylobacter jejuni, Cells Tissues Organs 26 (98) (2015) 90–98.
[38] A. Elmi, et al., Campylobacter jejuni outer membrane vesicles play an important role in bacterial interactions with human intestinal epithelial cells, Infect. Immun. 80 (12) (2012) 4089–4098.
[39] T.T. Tan, M. Mörgelin, A. Forsgren, K. Riesbeck, *Haemophilus influenzae* survival during complement-mediated attacks is promoted by *Moraxella catarrhalis* outer membrane vesicles, J. Infect. Dis. 195 (11) (2007) 1661–1670.
[40] M. Ferrer-Navarro, G. Torrent, E. Mongiardini, O. Conchillo-Solï¿½, I. Gibert, X. Daura, Proteomic analysis of outer membrane proteins and vesicles of a clinical isolate and a collection strain of Stenotrophomonas maltophilia, J. Proteom. 142 (2015) 122–129.
[41] O.Y. Kim, J. Lee, Y.S. Gho, Extracellular vesicle mimetics: novel alternatives to extracellular vesicle-based theranostics, drug delivery, and vaccines, Semin. Cell Dev. Biol. 67 (2017) 74–82.
[42] M. Toyofuku, N. Nomura, L. Eberl, Types and origins of bacterial membrane vesicles, Nat. Rev. Microbiol. 17 (1) (2019) 13–24.
[43] H. Owji, N. Nezafat, M. Negahdaripour, A. Hajiebrahimi, Y. Ghasemi, A comprehensive review of signal peptides: structure, roles, and applications, Eur. J. Cell Biol. 97 (6) (2018) 422–441.
[44] M. Paetzel, A. Karla, N.C.J. Strynadka, R.E. Dalbey, Signal peptidases, Chem. Rev. 102 (12) (2002) 4549–4579.
[45] M. Edman, T. Jarhede, M. Sjöström, Å. Wieslander, Different sequence patterns in signal peptides from mycoplasmas, other gram-positive bacteria, and Escherichia coli: a multivariate data analysis, Proteins Struct. Funct. Genet. 35 (2) (1999) 195–205.
[46] S. Hamsanathan, T.S. Anthonymuthu, U.K. Bageshwar, S.M. Musser, A hinged signal peptide hairpin enables tat-dependent protein translocation, Biophys. J. 113 (12) (2017) 2650–2668.
[47] R.A. Corey, W.J. Allen, I. Collinson, Protein translocation: what's the problem? Biochem. Soc. Trans. 44 (3) (2016) 753–759.
[48] E. Papanikou, S. Karamanou, A. Economou, Bacterial protein secretion through the translocase nanomachine, Nat. Rev. Microbiol. 5 (11) (2007) 839–851.
[49] M. Sandkvist, W.G.J. Hol, K.V. Korotkov, The type II secretion system: biogenesis, molecular architecture and mechanism, Nat. Rev. Microbiol. 10 (5) (2012) 336–351.
[50] J.M. Crane, L.L. Randall, The Sec system: protein export in Escherichia coli, EcoSal Plus 7 (2) (2017) 1–73.
[51] Y. Kato, K.I. Nishiyama, H. Tokuda, Depletion of SecDF-YajC causes a decrease in the level of SecG: implication for their functional interaction, FEBS Lett. 550 (1–3) (2003) 114–118.
[52] T. Bornemann, J. Jöckel, M.V. Rodnina, W. Wintermeyer, Signal sequence-independent membrane targeting of ribosomes containing short nascent peptides within the exit tunnel, Nat. Struct. Mol. Biol. 15 (5) (2008) 494–499.
[53] R. Kudva, K. Denks, P. Kuhn, A. Vogt, M. Müller, H.G. Koch, Protein translocation across the inner membrane of Gram-negative bacteria: the Sec and Tat dependent protein transport pathways, Res. Microbiol. 164 (6) (2013) 505–534.
[54] C. Robinson, et al., Transport and proofreading of proteins by the twin-arginine translocation (Tat) system in bacteria, Biochim. Biophys. Acta Biomembr. 1808 (3) (2011) 876–884.
[55] F. Sargent, N.R. Stanley, B.C. Berks, T. Palmer, T. Of, "Sec-independent protein translocation in Escherichia coli arginine " motif . The Tat system involves the integral,", 274 (51) (1999) 36073–36082.
[56] M. Müller, Twin-arginine-specific protein export in Escherichia coli, Res. Microbiol. 156 (2) (2005) 131–136.
[57] S. Zoufaly, et al., Mapping precursor-binding site on TatC subunit of twin arginine-specific protein translocase by site-specific photo cross-linking, J. Biol. Chem. 287 (16) (2012) 13430–13441.
[58] J.H. Campos, R.P. Soares, K. Ribeiro, A. Cronemberger Andrade, W.L. Batista, A.C. Torrecilhas, Extracellular vesicles: role in inflammatory responses and potential uses in vaccination in cancer and infectious diseases, J. Immunol. Res. 2015 (Figure 2) (2015) 2015.
[59] K.E. Bonnington, M.J. Kuehn, Protein selection and export via outer membrane vesicles, 6 (8) (2014) 247–253.
[60] K.S. Park, et al., Outer membrane vesicles derived from Escherichia coli induce systemic inflammatory response syndrome, PloS One 5 (6) (2010) 1–6.
[61] B. Shah, C.J. Sullivan, N.E. Lonergan, S. Stanley, M.C. Soult, L.D. Britt, Circulating

bacterial membrane vesicles cause sepsis in rats, Shock 37 (6) (2012) 621–628.
[62] J.H. Kim, et al., Outer membrane vesicles derived from Escherichia coli up-regulate expression of endothelial cell adhesion molecules in vitro and in vivo, PloS One 8 (3) (2013) 1–10.
[63] S.W. Hong, et al., Extracellular vesicles derived from Staphylococcus aureus induce atopic dermatitis-like skin inflammation, Allergy Eur. J. Allergy Clin. Immunol. 66 (3) (2011) 351–359.
[64] M.R. Kim, et al., Staphylococcus aureus-derived extracellular vesicles induce neutrophilic pulmonary inflammation via both Th1 and Th17 cell responses, Allergy Eur. J. Allergy Clin. Immunol. 67 (10) (2012) 1271–1281.
[65] D.R. Buelow, J.E. Christensen, J.M. Neal-Mckinney, M.E. Konkel, Campylobacter jejuni survival within human epithelial cells is enhanced by the secreted protein CiaI, Mol. Microbiol. 80 (5) (2011) 1296–1312.
[66] D. Bumann, et al., Proteome analysis of secreted proteins of the gastric pathogen Helicobacter pylori proteome analysis of secreted proteins of the gastric pathogen Helicobacter pylori, Infect. Immun. 70 (7) (2002) 3396–3403.
[67] Q. Luo, et al., Enterotoxigenic escherichia coli secretes a highly conserved mucin-degrading metalloprotease to effectively engage intestinal epithelial cells, Infect. Immun. 82 (2) (2014) 509–521.
[68] M.A. Croxen, B.B. Finlay, Molecular mechanisms of Escherichia coli pathogenicity, Nat. Rev. Microbiol. 8 (1) (2010) 26–38.
[69] W. Deng, et al., Quantitative proteomic analysis of type III secretome of enteropathogenic *Escherichia coli* reveals an expanded effector repertoire for attaching/effacing bacterial pathogens, Mol. Cell. Proteomics 11 (9) (2012) 692–709.
[70] A. Naz, et al., Identification of putative vaccine candidates against Helicobacter pylori exploiting exoproteome and secretome: a reverse vaccinology based approach, Infect. Genet. Evol. 32 (2015) 280–291.
[71] L.D.W. Luu, S. Octavia, L. Zhong, M. Raftery, V. Sintchenko, R. Lan, Characterisation of the Bordetella pertussis secretome under different media, J. Proteom. 158 (2017) 43–51.
[72] L.G. Pacheco, et al., A combined approach for comparative exoproteome analysis of Corynebacterium pseudotuberculosis, BMC Microbiol. 11 (2011).
[73] V. Mariappan, K.M. Vellasamy, J.S. Thimma, O.H. Hashim, J. Vadivelu, Identification of immunogenic proteins from Burkholderia cepacia secretome using proteomic analysis, Vaccine 28 (5) (2010) 1318–1324.
[74] P. Cabrita, et al., A secretome-based methodology may provide a better characterization of the virulence of Listeria monocytogenes: preliminary results, Talanta 83 (2) (2010) 457–463.
[75] S. Enany, Y. Yoshida, S. Magdeldin, Y. Zhang, X. Bo, T. Yamamoto, Extensive proteomic profiling of the secretome of European community acquired methicillin resistant Staphylococcus aureus clone, Peptides 37 (1) (2012) 128–137.
[76] C.W. Choi, et al., Analysis of Streptococcus pneumoniae secreted antigens by immuno-proteomic approach, Diagn. Microbiol. Infect. Dis. 72 (4) (2012) 318–327.
[77] Z. Wu, W. Zhang, C. Lu, Comparative proteome analysis of secreted proteins of Streptococcus suis serotype 9 isolates from diseased and healthy pigs, Microb. Pathog. 45 (3) (2008) 159–166.
[78] D.K. Tripathi, K. Srivastava, K.L. Nagpal, P.K. Shukla, K.K. Srivastava, Exploration of some new secretory proteins to be employed for companion diagnosis of Mycobacterium tuberculosis, Immunol. Lett. 209 (January) (2019) 67–74.
[79] P. Gaurivaud, et al., Mycoplasmas are no exception to extracellular vesicles release: revisiting old concepts, PloS One 13 (11) (2018) 1–14.
[80] H. Zhang, et al., Mycoplasma bovis MBOV_RS02825 encodes a secretory nuclease associated with cytotoxicity, Int. J. Mol. Sci. 17 (5) (2016).
[81] F.A. Khan, et al., Immunoproteomic identification of MbovP579, a promising diagnostic biomarker for serological detection of Mycoplasma bovis infection, Oncotarget 7 (26) (2016).
[82] M.A. Rasheed, et al., Comparative genomics of mycoplasma bovis strains reveals that decreased virulence with increasing passages might correlate with potential virulence-related factors, Front. Cell. Infect. Microbiol. 7 (May) (2017) 1–14.
[83] J.H. Fong, A. Marchler-Bauer, Protein subfamily assignment using the conserved domain database, BMC Res. Notes 1 (2008) 2–7.
[84] K. Sachse, C. Grajetzki, R. Rosengarten, I. Hänel, M. Heller, H. Pfützner, Mechanisms and factors involved in Mycoplasma bovis adhesion to host cells, Zentralbl. Bakteriol. 284 (1) (1996) 80–92.
[85] N.K. Wawegama, G.F. Browning, A. Kanci, M.S. Marenda, P.F. Markham, Development of a recombinant protein-based enzyme-linked immunosorbent assay for diagnosis of mycoplasma bovis infection in cattle, Clin. Vaccine Immunol. 21 (2) (2014) 196–202.
[86] C. Song, A. Kumar, M. Saleh, Bioinformatic comparison of bacterial secretomes, Dev. Reprod. Biol. 7 (1–2) (2009) 37–46.
[87] X. Zou, Y. Li, Y. Wang, Y. Zhou, Y. Liu, J. Xin, Molecular cloning and characterization of a surface-localized adhesion protein in mycoplasma bovis hubei-1 strain, PloS One 8 (7) (2013) 1–7.
[88] A. Behrens, F. Poumarat, D. Le Grand, M. Heller, R. Rosengarten, A newly identified immunodominant membrane protein (pMB67) involved in Mycoplasma bovis surface antigenic variation, Microbiology 142 (9) (1996) 2463–2470.
[89] P. Robino, et al., Genetic and antigenic characterization of the surface lipoprotein P48 of Mycoplasma bovis, Vet. Microbiol. 109 (3–4) (2005) 201–209.
[90] P. Fu, et al., Development of a direct competitive ELISA for the detection of Mycoplasma bovis infection based on a monoclonal antibody of P48 protein, BMC Vet. Res. 10 (2014) 1–8.
[91] A. Foddai, et al., Rapid differential diagnosis of Mycoplasma agalactiae and Mycoplasma bovis based on a multiplex-PCR and a PCR-RFLP, Mol. Cell. Probes 19 (3) (2005) 207–212.
[92] A. Thomas, et al., The p40*adhesin pseudogene of Mycoplasma bovis, Vet. Microbiol. 104 (3–4) (2004) 213–217.
[93] I. Lysnyansky, D. Yogev, S. Levisohn, Molecular characterization of the Mycoplasma bovis p68 gene, encoding a basic membrane protein with homology to P48 of Mycoplasma agalactiae, FEMS Microbiol. Lett. 279 (2) (2008) 234–242.
[94] A. Thomas, et al., Adherence to various host cell lines of Mycoplasma bovis strains differing in pathogenic and cultural features, Vet. Microbiol. 91 (2–3) (2003) 101–113.
[95] X. Chen, et al., P27 (MBOV_RS03440) is a novel fibronectin binding adhesin of Mycoplasma bovis, Int. J. Med. Microbiol. 27 (July) (2018) 0–1.
[96] B. Scherm, G.F. Gerlach, M. Runge, Analysis of heat shock protein 60 encoding genes of mycoplasmas and investigations concerning their role in immunity and infection, Vet. Microbiol. 89 (2–3) (2002) 141–150.
[97] A. Voros, A. Dunnett, L.G. Leduc, M.T. Saleh, Depleting proteins from the growth medium of Mycoplasma capricolum unmasks bacterium-derived enzymatic activities, Vet. Microbiol. 138 (3–4) (2009) 384–389.
[98] H.J. Wu, A.H.J. Wang, M.P. Jennings, Discovery of virulence factors of pathogenic bacteria, Curr. Opin. Chem. Biol. 12 (1) (2008) 93–101.
[99] D. Caccia, M. Dugo, M. Callari, I. Bongarzone, Bioinformatics tools for secretome analysis, Biochim. Biophys. Acta Protein Proteonomics 1834 (11) (2013) 2442–2453.
[100] D. Caccia, M. Dugo, M. Callari, I. Bongarzone, Bioinformatics tools for secretome analysis, Biochim. Biophys. Acta Protein Proteonomics 1834 (11) (2013) 2442–2453.
[101] K. Eichelbaum, J. Krijgsveld, Rapid temporal dynamics of transcription, protein synthesis, and secretion during macrophage activation, Mol. Cell. Proteomics 13 (3) (2014) 792–810.
[102] K. Eichelbaum, M. Winter, M.B. Diaz, S. Herzig, J. Krijgsveld, Selective enrichment of newly synthesized proteins for quantitative secretome analysis, Nat. Biotechnol. 30 (10) (2012) 984–990.
[103] A.R. Santos, et al., The Corynebacterium pseudotuberculosis in silico predicted pan-exoproteome, BMC Genom. 13 (Suppl 5) (2012) S6.
[104] Y. Yu, J. Champer, J. Kim, Analysis of the surface, secreted, and intracellular proteome of Propionibacterium acnes, EuPA Open Proteomics 9 (2015) 1–7.
[105] J.D. Bendtsen, L. Kiemer, A. Fausbøll, S. Brunak, Non-classical protein secretion in bacteria, BMC Microbiol. 5 (2005) 1–13.
[106] Y. Wang, et al., Site-specific cleavage of bacterial MucD by secreted proteases mediates antibacterial resistance in Arabidopsis, Nat. Commun. 10 (1) (2019).
[107] C. De Marcos Lousa, et al., Subcellular localization and trafficking of phytolongins (non-SNARE longins) in the plant secretory pathway, J. Exp. Bot. 67 (9) (2016) 2627–2639.