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Review Article

Bacterial Secretion Systems: Mechanisms, Functions and Biomedical Applications

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Abstract

Bacterial protein secretion refers to the process by which proteins are transported across the bacterial membrane to their extracytoplasmic site of action. This process is crucial for establishing a successful pathogen-host relationship, as proteins located on the bacterial surface or released into the extracellular environment play key roles in interacting with host cells. These secretion pathways are essential for pathogenesis, enabling bacteria to adhere to, invade, and manipulate host cellular processes to promote infection and survival.This is a problem because proteins need to pass through both the cytoplasmic and outer membranes in Gram-negative bacteria and the cytoplasmic membrane in Gram-positive bacteria. There are eight main kinds of secretion systems in Gram-negative bacteria, and each one has distinct structural and functional traits. One-step translocators, including the Type I, Type III, Type IV, and Type VI secretion systems, which directly transfer effectors into the target cells or environment, can be used to broadly classify these systems. On the other hand, before translocation, some systems, such as Type II and Type V, need to be exported to the periplasm through the Sec or Tat pathways.

These systems secreted proteins are essential for both environmental adaptability and bacterial pathogenicity. They may operate as adhesions that encourage bacterial colonization, poisons, or effector proteins that alter host cell functions. We now have a better knowledge of these intricate networks thanks to recent developments in genomic sequencing, which have also shown their evolutionary importance and their uses in industry and medicine. For example, focusing on the processes of bacterial secretion presents viable approaches to creating new treatments for infectious disorders. In order to clarify their modes of action and their consequences for both health and disease.

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1. Introduction:

Gram-negative bacteria are enclosed by two membranes: the inner and outer membranes. The peptidoglycan is a polymeric glycopeptide network that spans the gap between these membranes, known as the periplasm. This net has a well-defined mesh size and is connected to the outer and inner membranes by diverse proteins found in Gram-negative organisms, resulting in an equal distance between the two membranes. Proteins that are to be carried to the outer membrane or the extracellular space must traverse different hurdles on their route; this process is handled by a multitude of highly specialized secretion systems, generally classed by Roman numerals from type I to type IX [1]. Numerous animal, human, and plant hosts are susceptible to pathogenesis and disease caused by bacterial molecular secretion systems. Secretion systems provide several vital functions, such as transporting proteins, nucleic acids, and small molecules, depending on the life cycle of the bacterium [1]. Bacterial secretion systems play a crucial role in their survival and growth by transporting proteins from the cytoplasm to the outer membrane, the surrounding environment, or even into recipient cells. These systems act as intricate nanomachines, enabling bacteria to compete for resources, establish dominance in their environment, and interact with hosts and other bacteria. Numerous studies have explored these transporters, highlighting their importance in bacterial competition and environmental interactions. Pathogenic bacteria, in particular, utilize various strategies to infect mammalian hosts, cause tissue damage, and evade immune responses. A key aspect of their tactics involves the release of proteins across phospholipid membranes. These secreted proteins serve multiple functions, such as enhancing adhesion to eukaryotic cells, scavenging nutrients from their surroundings, and directly disrupting or modifying the functions of target host cells.To achieve these goals, many bacterial pathogens rely on specialized protein secretion systems to deliver virulence factors either into host cells or the external environment. These systems can be categorized based on their structure, function, and target specificity, reflecting their adaptability and precision in facilitating bacterial pathogenicity. [2-4].

2. The secretion of proteins by Gram-negative bacteria:

Many Gram-negative pathogenic bacteria use specialized secretion systems to transfer virulence proteins outside the cell and, in some cases, directly into the cytoplasm of eukaryotic or prokaryotic target cells. This process poses a significant challenge, as the secreted proteins must traverse two, and sometimes three, phospholipid membranes to reach their destination.In some cases, protein secretion in Gram-negative bacteria occurs in two distinct stages. Initially, the Sec or Tat secretion systems transport proteins to the periplasm. Subsequently, a Sec- or Tat-independent secretion mechanism facilitates the release of many other proteins through channels that span both the inner and outer membranes.Gram-negative bacteria possess specialized secretion systems, designated as Types I through XI, each responsible for secreting specific subsets of proteins. While these systems share the common feature of using β-barrel channels to form a ring in the bacterial outer membrane, they exhibit considerable diversity in their structural organization and molecular functions, allowing them to perform a wide range of roles in bacterial physiology and pathogenesis [2].

3. Mechanisms of Bacterial Secretion Systems:

3.1 Type I Secretion System (T1SS):

Gram-negative bacteria commonly have type I secretion systems, which enable the one-step transfer of a variety of proteins important for bacterial pathogenicity and nutrition acquisition, among other functions. The C-terminal secretion sequence of the majority of substrates with type I secretion systems is not broken either during or after translocation. Moreover, as figure 1 illustrates, these protein secretion nanomachines are always composed

of an ABC transporter, a membrane fusion protein (and both are present in the inner bacterial membrane), and an outer membrane protein. The periplasmic membrane's 'tunnel channel' is formed by these three membrane proteins when they come into contact with the substrate [5].

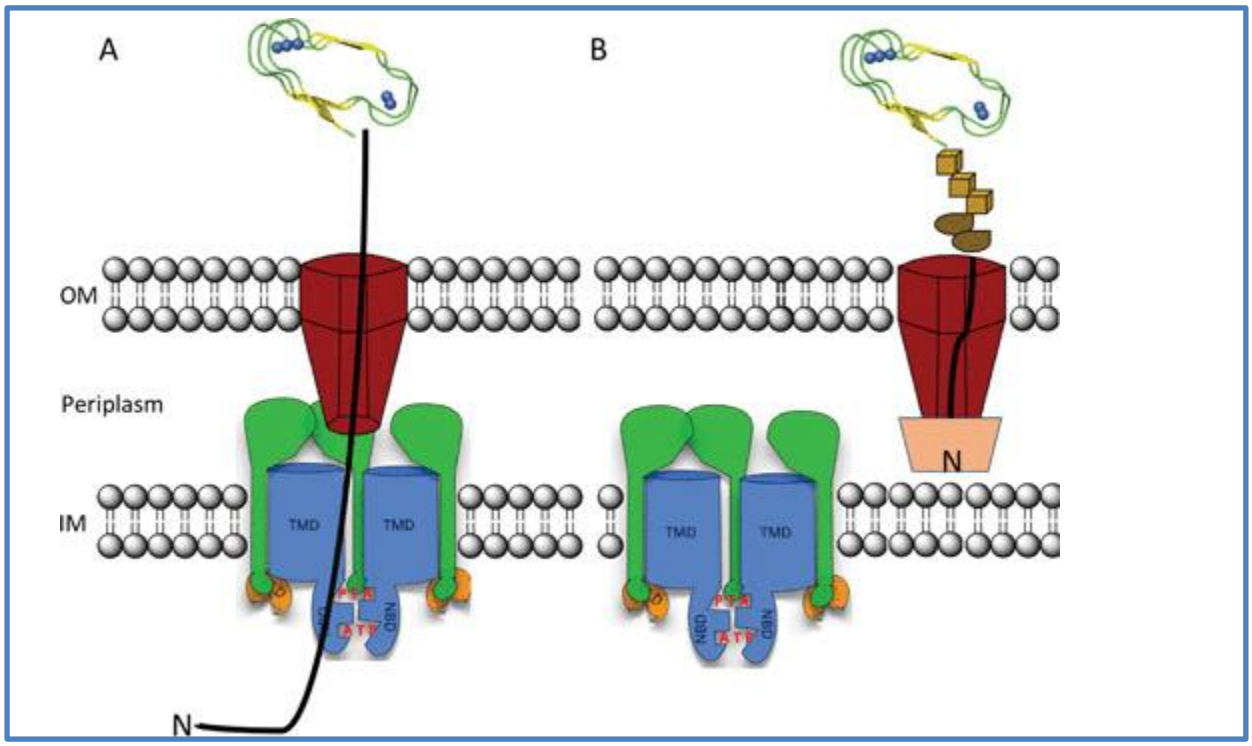

Figure 1. Schematic diagram of type I secretion system (A) and the recently identified mechanism of secretion for certain RTX adhesins, which results in a pseudoperiplasmic intermediate (B) and a so-called two-step process where secretion stalls just before completion. The OM protein appears in maroon, while the ABC transporter and MFP are displayed in blue and green, respectively. [6]

The C-terminal secretion sequence and non-peptide repeats, or "GG repeats," that are situated N terminal to the secretion sequence are the two main characteristics of T1SS substrates. The entire protein folds as a result of these GG repeats' binding to Ca^{2+} ions in the extracellular environment. Here, we provide an overview of what we now know about the secretion of these substrates by Gram-negative bacteria, which can have molecular masses of up to 1,500 kDa [7]. Protein Export Mechanism in the T1SS functions in three steps including [8].

1. **Recognition of the substrate**: The ABC transporter identifies the substrate protein and attaches itself to its C-terminal signal sequence [9]
2. **ATP hydrolysis**: When the substrate binds, ATP is hydrolyzed, releasing the energy required for protein translocation.
3. **Protein translocation**: The substrate protein is moved between the periplasmic space and inner membrane by use of the energy released during ATP hydrolysis. By joining the outer membrane protein and inner membrane ATPase, the MFP makes this process easier.

Passage through the outer membrane: The substrate protein is then moved into the extracellular environment via the pore that the outer membrane protein has created [10,11].

The T1SS is essential for both the virulence and antibiotic resistance of numerous bacterial species [7]. It participates in the release of a number of virulence factors, such as adhesins, poisons, and enzymes, which help bacteria invade host tissues, elude the immune system, and cause illness [12].Moreover, the β-lactamases, which are enzymes that break down β-lactam antibiotics, can be secreted by the T1SS. This leads to the emergence of antibiotic resistance, which complicates the management of bacterial infections [13].

3.2 Type II secretion system (T2SS):

Many bacteria utilize a multi-protein complex known as the Type II Secretion System (T2SS) to transport substrates across their cell membrane. These substrates, once released into the environment, serve as both local and long-range effectors, playing crucial roles in nutrient uptake, biofilm formation, and the promotion of pathogenicity. The T2SS has gained recognition as a key contributor to virulence in both animal and plant pathogens.The T2SS spans the bacterial cell envelope and extrudes substrates through a secretin channel located in the outer membrane, utilizing a structure called the pseudopilus. The assembly and function of the pseudopilus are regulated by an inner membrane assembly platform and a cytoplasmic motor, which together coordinate the precise extrusion of substrates critical for bacterial survival and interaction with their environment. [14,15].

As illustrated in Figure 2, the Type II Secretion System (T2SS) is divided into three distinct subcomplexes that work together to facilitate substrate secretion. The outer membrane complex (OMC) includes the secretin channel, formed by the general secretory protein (GspD), and its associated pilotin, general secretory protein (GspS), which enables the extrusion of substrates through the outer membrane. The inner membrane complex, also known as the assembly platform (AP), consists of several components, including the cytoplasmic ATPase general secretory protein (GspE) and the pseudopilins general secretory protein (GspC, GspF, GspL, and GspM), which coordinate the assembly of the pseudopilus and provide energy for secretion. The pseudopilus itself is composed of the major pseudopilin GspG and the minor pseudopilins GspGHIJK, which together facilitate substrate movement. The pseudopilins are processed by the inner membrane-embedded prepilin peptidase GspO, which prepares them for assembly into the pseudopilus, ensuring efficient substrate extrusion through the T2SS [16]. Additionally, a membrane protein called general secretory protein N, a part of the AP in the inner membrane, is present in some T2SS. Additionally, certain bacteria encode general secretory protein A and general secretory protein B, two inner membrane peptidoglycan-interacting proteins that help locate general secretory protein (GspD) on the outer membrane [17]. The Sec or Tat systems are responsible for the initial transportation of T2SS substrates across the inner membrane [18].

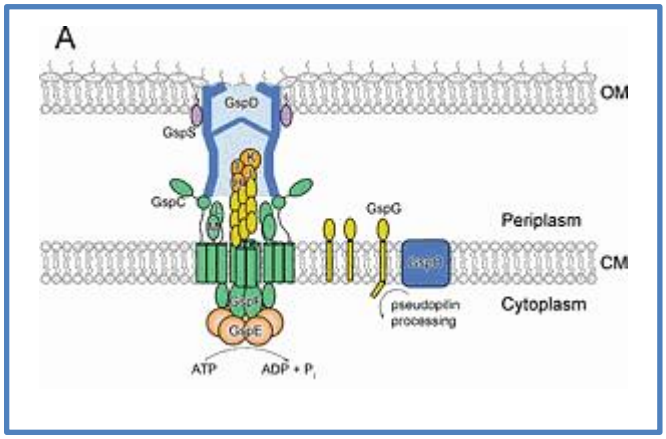

Figure .2: An illustration showing the location and topology of the T2SS's conserved core components [19]

The substrate is subsequently targeted for secretion from the periplasm via a variety of different, and as yet poorly understood mechanisms that seem distinct to each substrate and lack a common signal sequence. Toxins, lipases, metalloproteases, and digestive enzymes are examples of T2SS substrates [19]. Since engagement with the T2SS requires folding of the T2SS substrates, a secretion signal is assumed to form in the folded structures; however, since T2SS substrate architectures differ widely, a common, universal secretion signal has not yet been discovered. Once the T2SS substrates enter the periplasmic compartment, it has been proposed that GspCs protein-protein interaction domain (PDZ) will identify and recruit them. Interactions between the Type II Secretion System (T2SS) substrates and key components of the system have also been observed. These include interactions with general secretory proteins (GspD) and the pseudopilus tip proteins (GspH, GspI, GspJ, and GspK). Additionally, substrates interact with the cytoplasmic membrane (CM) proteins GspL and GspM, highlighting the coordinated roles of these proteins in substrate recognition, processing, and transport through the T2SS machinery[20]. These interactions are broadly consistent with the two most popular ideas for pushing proteins through the secretin pore: the piston mechanism and the Archimedes screw [21]. The piston model suggests that the pseudopilus tip drives substrates through the secretin channel in a linear, pushing motion. However, this model falls short in explaining the retraction of the pseudopilus and the recharging of substrates, as the Type II Secretion System (T2SS) lacks a dedicated retraction ATPase. This limitation highlights the need for alternative mechanisms or additional factors to fully account for the dynamic assembly and disassembly processes within the T2SS[22]. Through rotating motion brought on by interactions with the poly-GspG shaft of the pseudopilus, the Archimedes screw model threads the T2SS substrates out through the secretin pore; nevertheless, this model requires ongoing GspG degradation and replenishment [23]. [23]. Degradation may result from the selective removal of GspG-bound calcium and the ensuing destabilization of GspG [24]; however, considering the dimensions of the pseudopilus, the rigid structure of the double-barrel secretin domain, and the increasing evidence for the pseudopilus's rotation, perhaps a composite model should be taken into consideration, in which the substrates secreted are driven out by the pseudopilus tip through its rotary motion.

According to recent research [25], the pathogen Acinetobacter baumannii secretes several T2SS substrates, including the lipases LipA and LipH and the protease CpaA . These substrates are necessary for the pathogenicity of A. baumannii. It's interesting to note that LipA and CpaA were associated with distinct periplasmic chaperones that were necessary for T2SS recruitment and secretion and were anchored in

the inner membrane, respectively, dubbed LipB [26] and CpaB [27]. According to bioinformatics, as shown in V. cholerae, P. aeruginosa, and Burkholderia pseudomallei, many T2SS effectors are really linked in dimeric operons with putative membrane-bound chaperones [28]. Toxins and a variety of hydrolytic enzymes, including as lipases, proteases, and enzymes that are active on carbohydrates, are sent to the extracellular space or cell surface of Gram-negative bacteria by the type II secretion system (T2SS) [19].

3.3 The type III secretion system (T3SS) :

The Type III Secretion System (T3SS) is a massive 3.5 MDa complex that spans the double membrane of Gram-negative bacteria and extends into the extracellular environment. Its assembly requires approximately 25 structural and auxiliary proteins. The T3SS is composed of several key components, including a cytoplasmic domain containing an ATPase, inner rings integrated into the membrane, a domain that traverses the periplasm, outer membrane rings, a hollow filament that links the bacterial cytoplasm to the target cell, and translocator proteins that form a pore in the target cell's plasma membrane.As illustrated in Figure 3, the T3SS can include a tip protein that regulates secretion, ensuring it only occurs upon contact with a host cell. In bacteria that interact with plants, the filament connecting the bacterial cytoplasm to the target cell is referred to as a pilus, whereas in bacteria that interact with other species, it is known as a needle. The outer membrane ring of the T3SS associates with secretins, which are also found in type II secretion systems and type IV pili.Proteins designated for secretion are first transported from the bacterial cytoplasm to the periplasm through Sec- or Tat-dependent pathways. From there, the type II secretion system facilitates their release into the extracellular environment through a multimeric pore complex called the secretin. This secretin-like structure is also a feature of type IV pili, which can transfer proteins or DNA into target cells, similar to the T3SS, or take up DNA from the surrounding environment. [29].

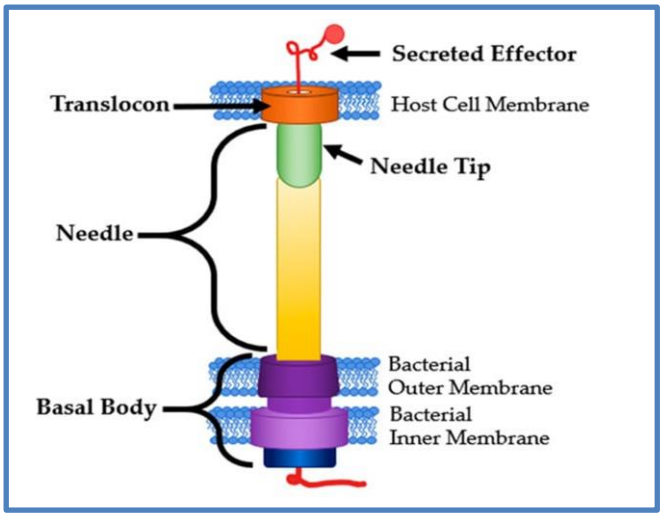

Figure. 3: T3SS Structure and Common Targets [29]

Type III secretion systems (T3SS) linked to virulence facilitate the introduction of bacterial effector proteins into eukaryotic host cells. They have developed to sense host cell contact and to inject their substrates through a translocon pore in the host cell membrane. They can produce a wide variety of substrate proteins to modify host cell activity. Type III Secretion System (T3SS) substrates typically possess an N-terminal signal sequence and a chaperone-binding domain, which facilitate proper interaction with T3SS-specific

chaperones. These signals guide the substrates to the secretion apparatus, where they are unfolded and directed into the secretion channel. This channel is formed by the needle filament and the transmembrane domains of the export apparatus components. The secretion process is powered by the proton motive force across the bacterial inner membrane, providing the energy needed to propel substrates through the T3SS[30].

3.4 Type IV secretion systems:

Many bacteria possess large protein complexes known as Type IV Secretion Systems (T4SSs), which traverse their cell membranes. These systems are equipped with a channel that facilitates the movement of proteins or protein-DNA complexes across the membrane. The translocation process is powered by several cytoplasmic ATPases, which drive significant conformational changes within the secretion complex, ensuring efficient transport.The versatility of T4SSs is evident in the diverse roles they perform. These systems are highly adaptable, enabling bacteria to engage in various activities, such as transferring genetic material, delivering effector proteins into host cells, and establishing interactions with their environment or other organisms. This adaptability highlights the evolutionary significance and functional diversity of the T4SS family. [31]. To transport DNA, proteins, or other macromolecules to bacterial or eukaryotic cell targets, a variety of bacterial species use Type IV Secretion Systems (T4SSs) [32]. The conjugation systems and effector translocators are the two subfamilies that make up the majority of the T4SSs [33]. Conjugation systems play a crucial role in the dissemination of mobile genetic elements (MGEs), which often carry genes for antibiotic resistance or heavy metal resistance. This ability to transfer resistance traits between bacterial populations is a significant medical concern, as it contributes to the rapid spread of drug-resistant pathogens and complicates the treatment of bacterial infections. [34]. While effector translocators primarily facilitate the transport of proteins into eukaryotic target cells, recent research has revealed their broader capabilities, including the interkingdom transfer of DNA, peptidoglycan, and other macromolecules. This expanded understanding highlights the diverse and complex interactions between bacteria and their hosts[35]. Recent studies have demonstrated that Gram-positive pathogens also utilize effector translocator systems, a feature previously recognized as critical to the pathogenicity of Gram-negative bacteria. While most conjugation systems and effector translocators rely on direct cell-to-cell contact for their function, some Type IV Secretion Systems (T4SSs) exhibit the unique ability to import external DNA or export DNA and proteins into the environment without requiring interaction with a target cell. This versatility underscores the diverse mechanisms employed by T4SSs in bacterial adaptation and survival. [37].

Significant progress has been made in understanding the structures and assembly pathways of Type IV Secretion Systems (T4SSs) in recent years. Structural studies began around 23 years ago with the publication of X-ray structures of a few highly conserved T4SS subunits. These early discoveries laid the foundation for subsequent research, which has since advanced our knowledge of the complex architecture and assembly processes of these essential bacterial systems[38]. In the years following the initial structural studies, single-particle electron microscopy and

crystallography were employed to resolve larger subassemblies, approximately 1 megadalton (MDa) in size, from the R388 and pKM101 plasmid conjugation systems. These advances provided detailed insights into the structural organization of Type IV Secretion Systems (T4SSs) and their components, further enhancing our understanding of their function and assembly[39]. Recent advancements in electron microscopy have revolutionized the study of Type IV Secretion Systems (T4SSs), enabling researchers to visualize intact T4SSs within their natural context in the bacterial cell envelope. These cutting-edge techniques provide a more comprehensive understanding of the structural organization and functionality of T4SSs as they operate within living bacterial cells[40].

Type IV Secretion Systems (T4SSs) are composed of a core group of conserved subunits. In Gram-negative bacteria, approximately 12 of these subunits are necessary to assemble fully functional "minimized" systems. This streamlined composition underscores the efficiency and adaptability of T4SSs in facilitating diverse bacterial processes[41]. A unified nomenclature for the Type IV Secretion System (T4SS) superfamily has been established based on the model Agrobacterium tumefaciens VirB/VirD4 system. According to this naming convention, the core subunits are designated as VirB1 through VirB11, along with VirD4 (as shown in Figure 4). This standardized terminology aids in the consistent classification and study of T4SS components across different bacterial species. [42]. The cytoplasmic energy center of the Type IV Secretion System (T4SS), positioned at the base of the translocation channel, is composed of three ATPases: VirD4, VirB4, and VirB11. Among these, VirD4 plays a crucial role by binding DNA and protein substrates before they are directed into the translocation channel, ensuring proper substrate processing and delivery[43]. The translocation channel of the Type IV Secretion System (T4SS) consists of two major subassemblies: one spanning the inner membrane (IM) and the other situated within the periplasm and outer membrane (OM). The inner membrane complex (IMC), which represents the minimal structural components required, is composed of VirB3, VirB6, VirB8, and the N-terminal region of VirB10. These elements form the foundational framework for the channel's operation within the inner membrane[44]._Another integral component of the inner membrane complex (IMC) is the VirB4 ATPase. This ATPase establishes a stable association with the translocation channel, contributing to its structural integrity and providing the energy necessary for substrate transfer through the system[36].The outer membrane core complex (OMCC), which is made up primarily of the lipoproteins VirB7, VirB9, and a C-terminal region of VirB10, is connected to the IMC by a stalk or cylinder [44]. The Type IV Secretion System (T4SS) channel must first attract substrates to its cytoplasmic entry site before transferring them across the cell membrane. This process begins with a group of processing factors known as DNA transfer and replication (Dtr) proteins. These proteins bind to the origin-of-transfer (oriT) sequence to form a complex called the relaxosome. The relaxosome initiates the recruitment of mobile genetic elements (MGEs) to their corresponding conjugation, or "mating," channels, ensuring precise substrate targeting and transfer [45].

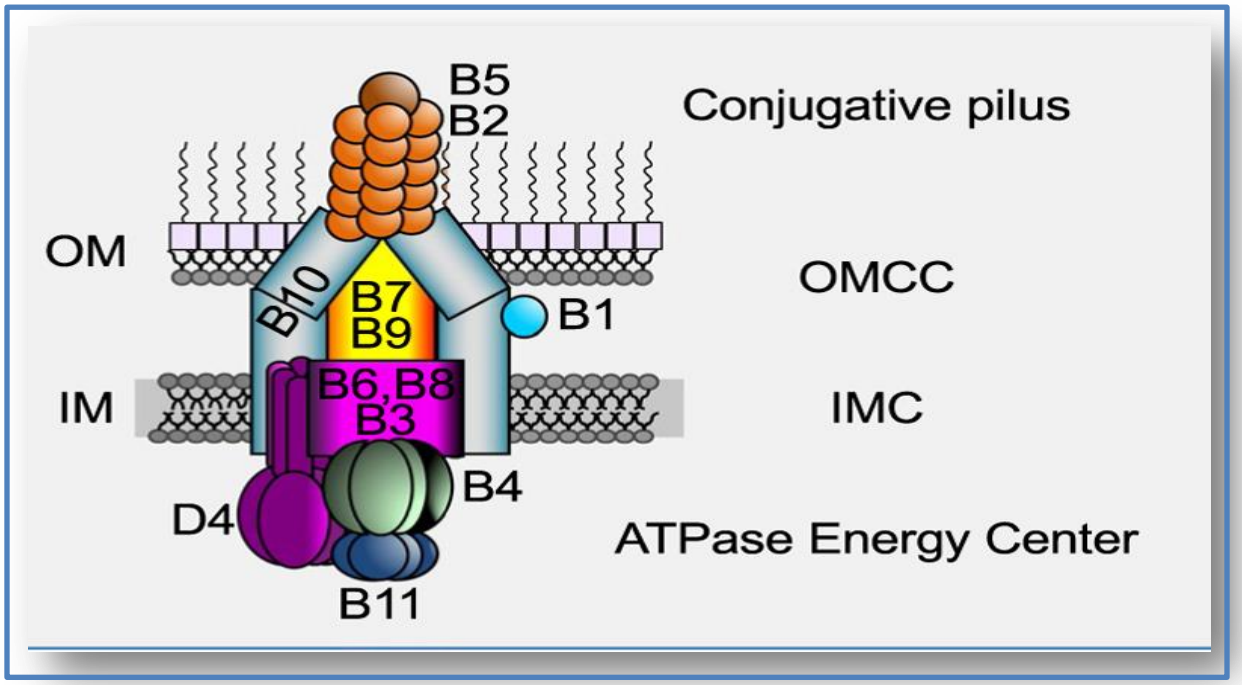

Figure no. 4: Locations of the inner membrane complex (IMC) and outer membrane core complex (OMCC) are shown in the animation, along with the corresponding VirB/VirD4 subunits. Right: Corresponding 3D reconstruction of the R388-encoded substructure made up of the VirB3 – VirB10 subunits, showing side and bottom views at 90° angles. Single-particle negative-stain electron microscopy (EMD-2567) was used to visualize the substructure; the two adjacent hexameric barrels of the VirB4 ATPase are shown in pink coloring.

3.5.Type V secretion system (T5SS):

Substrates of the Type V Secretion System (T5SS) are unique in that they can secrete spontaneously, unlike other secretion systems that require specialized apparatuses or membrane channels to transport substrates across the bacterial membrane. These substrates possess a β-barrel domain that inserts itself into the outer membrane, forming a channel. This channel facilitates the transport of the remainder of the protein or another associated protein, enabling efficient secretion without the need for additional complex machinery[46,47]. Proteins secreted by the Type V Secretion System (T5SS) must first be translocated across the inner membrane into the periplasm in an unfolded state by the Sec apparatus. This initial step is essential, as T5SSs operate exclusively in the outer membrane. During this process, the N-terminal Sec signal sequence of the T5SS proteins is cleaved off as they enter the periplasm, preparing them for subsequent folding and secretion through the outer membrane[48].

Type V Secretion Systems (T5SSs) are categorized into several sub-classes based on their structural features and domain organization (as illustrated in Figure 5). Among these, Type V autotransporters (ATs) are grouped into sub-classes Va, Ve, and the recently proposed Vf. While this sub-classification, based on domain structure, highlights differences in their organization and biogenesis mechanisms, it does not always

correspond to the functional roles of the secreted passenger proteins.Passenger proteins secreted by Type V ATs serve diverse functions, acting as adhesins to facilitate bacterial attachment, enzymes to catalyze specific reactions, or toxic proteins that contribute to pathogenicity. This functional diversity underscores the versatility and adaptability of Type V secretion systems in bacterial biology[49].

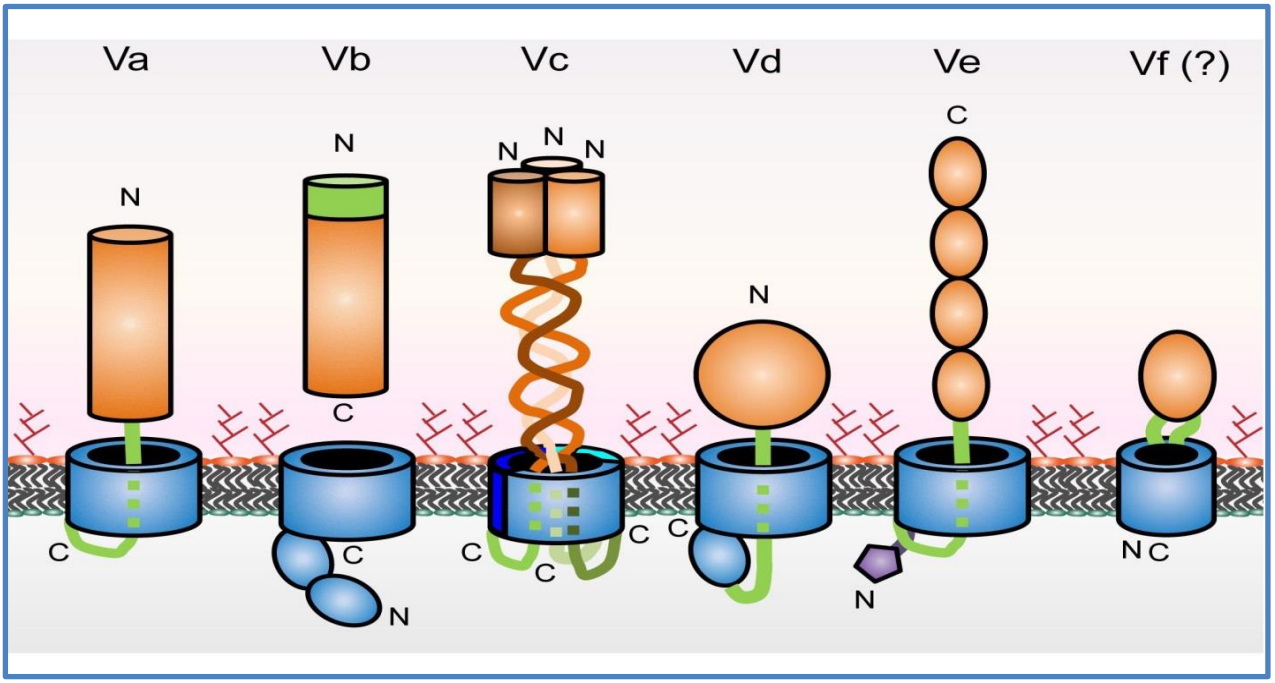

Figure no.5: This schematic highlights both the shared and unique features across subclasses, providing insights into their structural and functional diversity. β-barrels and POTRA domains are shown in blue, linkers and TPS domains in green, and passenger domains in orange. The periplasmic extension characteristic of Type Ve proteins is depicted in purple. The locations of the N- and C-termini are labeled for orientation. The inclusion of Type Vf is tentative, as indicated by the question mark, reflecting its uncertain classification within the Type V Secretion System.

3.6 The type VI secretion systems (T6SS):

Type VI Secretion Systems (T6SS) are present in approximately 25% of all Gram-negative bacteria. These systems share evolutionary similarities with contractile nanomachines such as bacteriophages and R-type pyocins. The assembly of the T6SS begins with the formation of a membrane complex that anchors a phage-like baseplate. This baseplate is connected to a sharp spike, which is then encased in a polymerized inner tube surrounded by an outer contractile sheath.During assembly, various mechanisms preload effector molecules onto the spike or tube. When the sheath contracts, it generates an immense amount of energy, propelling the spike and tube, along with their associated effectors, out of the effector cell and into bacterial or eukaryotic target cells. A specialized T6SS-specific unfoldase recycles the contracted sheath subunits, enabling the system to reassemble and fire again.Live-cell imaging has shown that T6SS assembly is highly dynamic, with precise

subcellular localization within specific bacteria. This remarkable accuracy ensures efficient deployment of the system. T6SS primarily contributes to bacterial pathogenicity and competitiveness by delivering effectors that disrupt target cells or confer an advantage in bacterial interactions [50].

3.7.The Type VII secretory system (T7SS):

The Type VII secretory system has been identified in species of *Mycobacterium*, *Corynebacterium*, and several Gram-positive bacteria, including Staphylococcus aureus. This SS, which is a significant virulence factor in Mycobacterium TB, was initially identified in 2003 in the bacteria known as ESX-1 [51]. There are now five T7SS known to exist in Mycobacterium species, but it is nearly impossible to determine how they are transported across the mycobacterial membrane . The EscAB clan, which includes six protein families—Esx, PE, PPE, LXG, DUF2563, and DUF2580—contains the majority of substrates for the Type VII Secretion System (T7SS). Among these, ESAT-6, a protein from Mycobacterium tuberculosis, is a notable member of the Esx family. ESAT-6 plays a significant role in the function and pathogenicity of T7SS, underscoring the importance of this protein family in bacterial processes[2,3,52].

3.8.The type IX secretion system (T9SS):

The most recent system to be identified is the Por secretion system (PorSS), also known as the type IX secretion system (T9SS) [53]. The Type IX Secretion System (T9SS) is responsible for transporting molecules across the outer membrane of bacteria. Its substrates must contain a Sec signal, which facilitates their transport across the inner membrane with the assistance of the Sec system. While T9SS has been identified in nearly all members of the Bacteroidetes phylum, it has been primarily studied in oral pathogens such as Tannerella forsythia and Porphyromonas gingivalis. In P. gingivalis, the T9SS consists of 16 proteins with structural and functional roles, along with two additional proteins that regulate the transport process, highlighting the system's complexity and importance in bacterial physiology and pathogenicity[53,54].

4. Protein Secretion by Gram-Positive Bacteria:

Gram-positive bacteria have a single lipid bilayer and a thick cell wall. Furthermore, some Gram-positive bacteria, particularly Mycobacteria, have a lipid-rich cell wall known as a mycomembrane. Because of these variations in fundamental cell structure, it is not unexpected that Gram-positive bacteria have different systems for secreting extracellular proteins than Gram-negative species do. Gram-positive bacteria, like Gram-negative species, transport proteins across the cytoplasmic membrane via the Tat and Sec routes. However, in many circumstances, this transit is insufficient to get proteins to their final destinations [2].

5. Functions of Bacterial Secretion Systems:

Bacterial molecular secretion systems play a central role in causing diseases and pathogenicity across a wide range of hosts, including animals, humans, and plants. These systems carry out diverse and critical functions depending on the bacteria's lifecycle, such as transferring small molecules, nucleic acids, and proteins. The interplay of these secretory pathways significantly shapes bacterial survival strategies and the mechanisms by which infections impact human, animal, and plant health.Understanding the molecular processes governing these secretion systems is vital for the development of effective antivirulence therapies and the treatment of bacterial infections. These systems are essential for bacterial biology, as they transport proteins from the cytoplasm to the outer membrane and from donor cells to the environment or recipient

cells. Over time, many of these transporters have evolved into sophisticated nanomachines that enable bacteria to compete for resources and space. Extensive research and reviews have explored how these systems promote bacterial interactions with the environment, other bacteria, and hosts, highlighting their importance in microbial ecology and pathogenesis[2-4].

5.1. Intersecretion system-mediated response in bacteria:

Bacterial secretion systems interact through various mechanisms, including collaborative assault, Intimate host-bacteria contact, exploitative competition, and horizontal gene transfer (HGT) play crucial roles in the dynamics of both plant and animal pathogens as well as non-pathogenic bacteria. These processes enable bacteria to interact closely with hosts, outcompete rivals, and exchange genetic material, facilitating adaptation and survival. Figure 1 provides an overview of these interaction-mediated functions, illustrating how bacteria leverage these mechanisms to thrive in diverse environments and influence their interactions with hosts and other microbial communities [55-57].

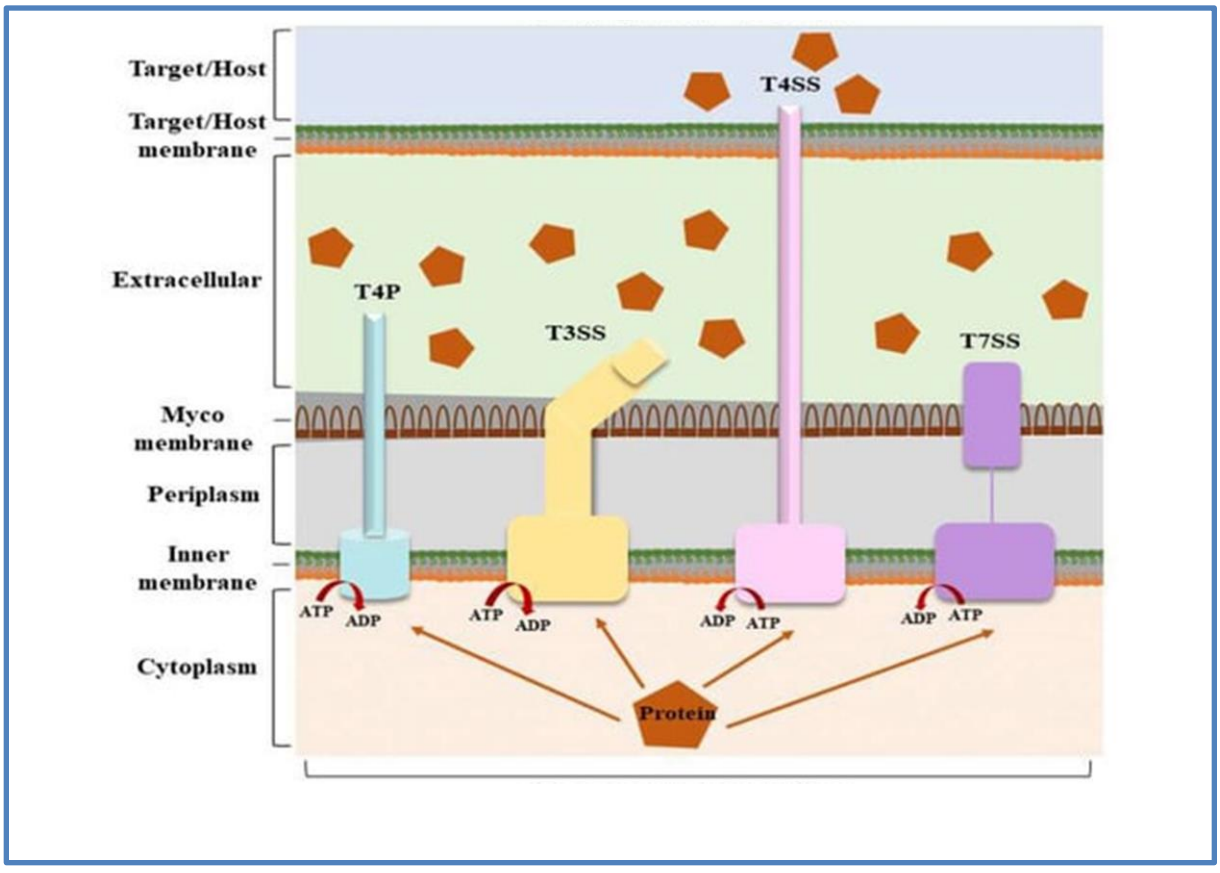

Figure 1. Bacterial secretion system interactions

5.2.Host cell attachment and damage are associated with effector translocation:

Bacterial contact with the host surface is crucial for establishing an infection and promoting pathogen internalization and life cycle progression [55,58].

The Type V Secretion System (T5SS) encompasses several distinct classes, each tailored for specific structural and functional roles in protein transport. These include classical autotransporters (T5aSS), which facilitate their own secretion, and two-partner secretion systems (T5bSS), where one protein forms a β-barrel to enable the secretion of a partner protein. Trimeric autotransporter adhesins (T5cSS) assemble into trimeric structures for adhesion purposes, while T5dSS represents a less-characterized subclass with unique features. Inverse autotransporters (T5eSS) differ in topology from classical autotransporters, and T5fSS is a recently proposed subclass that remains to be fully characterized. This diversity illustrates the adaptability of T5SS in fulfilling a variety of bacterial functions[59]. Several T5SS classes work with T3SS and T4SS to establish interaction between pathogens and host adhesin receptors, leading to cytoskeletal changes and host cell invasion [60].

5.3. Contact between microbes mediated by the intersection system:

Recent studies have revealed that interactions between the Type VI Secretion System (T6SS) and the Type IV Secretion System (T4SS) play a significant role in mediating contact between Neisseria cinerea, a non-pathogenic bacterial species, and other human pathogens. These interactions highlight the complex dynamics within microbial communities, where T6SS and T4SS may influence bacterial competition, horizontal gene transfer, and the modulation of host-pathogen interactions. This discovery underscores the importance of secretion system interplay in shaping both non-pathogenic and pathogenic bacterial behaviors in human-associated environments [56]. As mentioned earlier, Type IV Pili (T4Ps) are integral components of the VirB/D4 Type IV Secretion System (T4SS) architecture. These pili are located on the bacterial surface, where they play a crucial role in facilitating close physical contact between bacteria and host cells, as well as interactions with other bacteria. This proximity is essential for processes such as adhesion, horizontal gene transfer, and the delivery of effector molecules, contributing to the overall functionality and adaptability of the bacterial secretion system[60 In competition experiments involving human commensal and pathogenic Neisseria strains, Neisseria cinerea demonstrated a remarkable ability to kill pathogenic strains such as N. meningitidis and N. gonorrhoeae. This effect was shown to be T6SS-dependent, highlighting the crucial role of the Type VI Secretion System in mediating bacterial competition and conferring a survival advantage to N. cinerea in microbial communities[61]. Prey strains of Neisseria that lacked Type IV Pili (T4P) were able to evade T6SS-mediated killing by segregating to the periphery of agar-seeded colonies, avoiding direct interaction with the predator strain. In contrast, prey strains that expressed pili were outcompeted by the killer strain, as the T4P facilitated cellular contact with N. cinerea, enabling the predator to effectively deploy its T6SS. This observation suggests that T4P promotes the use of contact-dependent T6SS by drawing prey bacteria closer to the predator.The ability of bacteria to influence the outcome of infections by directly killing competitors using the antibacterial properties of the T6SS is well-documented. This mechanism not only alters microbial population dynamics but also has broad ecological implications, shaping microbial communities and influencing host-pathogen interactions [62]. As a result, the interplay between secretion systems, facilitated by pilus-mediated contact, may have a profound influence on microbial community structures and composition. This interaction mirrors the impact of the T6SS, which can alter microbial populations through its antibacterial properties. By promoting direct contact, pili

enable more effective deployment of contact-dependent systems like the T6SS, thereby shaping competitive dynamics and ecological relationships within microbial communities[62].

6. Biomedical Applications of Bacterial Secretion Systems:

Bacterial secretion systems are increasingly being utilized as innovative strategies for biotherapeutic delivery. These systems can secrete a variety of products, including recombinant proteins with or without fusion to carrier proteins. The natural secretion mechanisms of bacteria (illustrated in Figure 6) are often employed for therapeutic delivery, preserving the integrity of bacterial cells and enabling microbes to maintain interactions with their hosts.Close physical contact between the bacterial delivery vehicles and host cells, particularly at the epithelial barrier, is thought to facilitate the diffusion of effector molecules. This interaction may help these molecules traverse gaps in tight junctions, promoting their systemic distribution. Such mechanisms highlight the potential of bacterial secretion systems in advancing targeted therapeutic delivery while maintaining host-microbe interaction dynamics[63]. While the exact mechanism of action is unknown,. found that oral administration of recombinant probiotic-secreting interleukin-22 (IL-22) to mice enhanced systemic IL-22 levels [64].

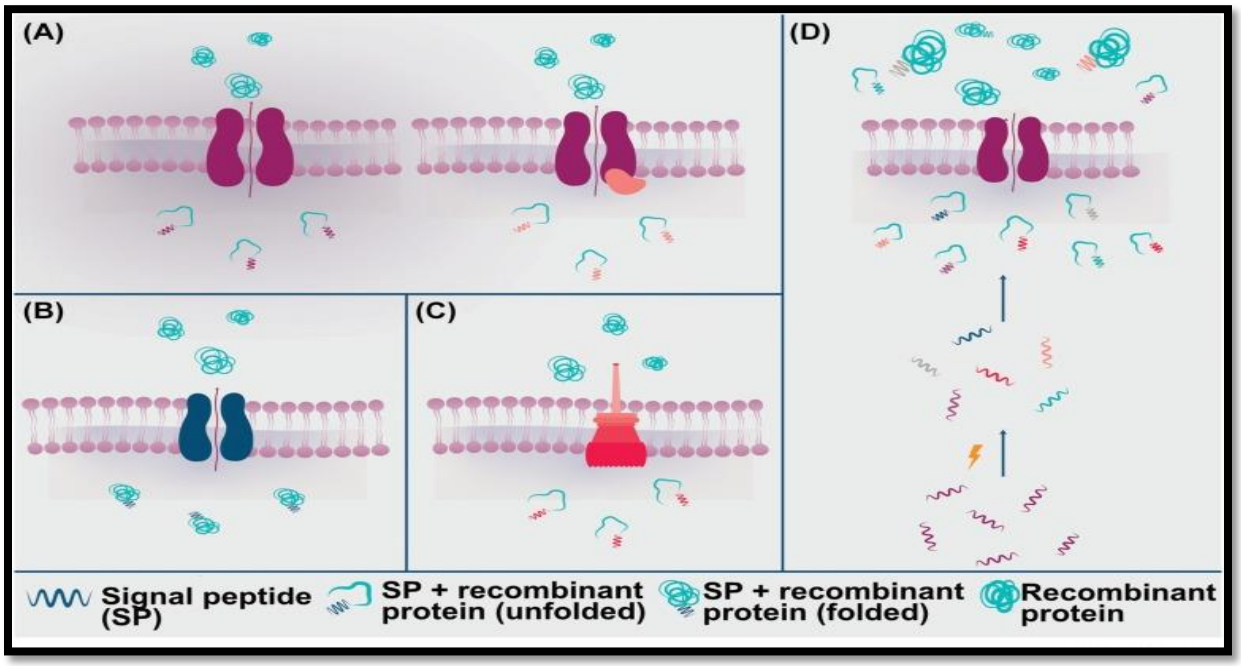

Figure 6: Secretory systems employed for biotherapeutic and vaccine administration. The most often used secretion systems for bacterial therapeutic administration are Sec (+/- SecA2), Tat, and Type III. Signal peptides direct unfolded (Sec, SecA2, and Type III) or folded (Tat) recombinant proteins to secretory machinery and are cleaved upon mature protein translocation into the extracellular space. (D) Signal peptide optimization is frequently necessary for the release of correctly cleaved and folded mature recombinant protein, posing a significant bottleneck for this route of administration. Native signal peptides, heterologous signal peptides, or mutagenesis (lightning bolt) can all be used to build signal peptide libraries. Finding a signal peptide that is correctly cleaved and causes the effective translocation of mature recombinant protein can be achieved by high-throughput screening of

these signal peptides linked to recombinant proteins.

Recombinant protein secretion from bacteria is not easily engineered. Because it burdens a vital mechanism for the organism, using the secretion machinery of the cell to release the recombinant protein is detrimental. Cell membrane synthesis, energy conversion, and food uptake are all carried out by the native secretory machinery [2]. Overexpression of recombinant proteins that take advantage of secretion mechanisms may lead to deficiencies in fitness [65]. This may have a detrimental effect on efficacy downstream by reducing delivery efficiency. Although synthetic gene circuits are frequently employed to regulate the expression of medicines, some promising research investigates ways to get around the fitness burden they impose [66].

Conclusions:

Understanding bacterial secretion systems provides insights into their roles and infection competition and adaption. This knowledge underpins advancement and in antivirulence and biotechnology innovation, offering novel strategies to combat bacterial infections and harness bacterial systems for the therapeutic delivery.

Biomedical applications leverage of secretion systems for the therapeutic delivery, including the secretion of recombinant proteins and vaccines. Signal peptide optimization and synthetic gene circuits are used to improve secretion efficiency, though challenges such as fitness burdens on bacterial hosts remain .promising research focuses on minimizing these drawbacks while enhancing therapeutic efficacy.

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