Pakistan J. Zool., vol. 55(3), pp 1457-1468, 2023 **DOI**: https://dx.doi.org/10.17582/journal.pjz/20220721050719

Review Article

Features and Prospect of Type V Secretion System in Bacteria

Meng Wang, Dongliang Zhou, Huixia Li, Chunqin Wu,Yan Zhao and Houqiang Luo*

College of Animal Science, Wenzhou Vocational College of Science and Technology, Wenzhou 325006, People's Republic of China

Article Information
Received 21 July 2022
Revised 15 August 2022
Accepted 19 September 2022
Available online 14 March 2023 (early access)
Published 19 April 2023

Authors' Contribution
MW wrote the paper. DLZ performed the visualization. MW, HXL, CQW, YZ, ZZS and HQL participated in the overall design and literature review. All authors revised the manuscript.

Key words
T5SS, Autotransporter, Bacteria, Structure, Function, Prospect

ABSTRACT

Bacteria use a variety of mechanisms to transport proteins synthesized in the cytoplasm to the outer membrane or extracellular environment of the bacterial cell, or directly to other cells. The bacterial secretion system plays an irreplaceable role in this process. Up to now, at least 11 kinds of secretion systems are involved in the pathogenesis role of bacteria, especially the formation of bacterial resistance. Therefore, the study of the bacterial secretion system is of great significance for antibiotic treatment of bacterial diseases. Most of the secretion systems identified so far are found in Gram-negative bacteria. There are a few secretion systems in Gram-positive bacteria, such as type VII secretion system, which is found in *Mycobacterium tuberculosis*. T5SS may be the simplest secretion system available among all the secretion systems, and plays a vital role in the pathogenic mechanism of bacteria by participating in bacterial adhesion, biofilm formation, and contributing to the ability of nutrition acquisition a nd environme ntal adaptability. T5SS is a vital factor in the gradual reduction of antibiotic effectiveness, so the system has been the target of alternative antimicrobial strategies based on small molecules and antibodies. This review mainly introduces the discovery history of the bacterial secretion system, structure, and function of T5SS, and summarizes its potential applications and existing issues. Presently we have not elucidated the specific pathogenic mechanism of this system, thus the intensive study is significant.

INTRODUCTION

The investigation has shown the existence of at least 11 secretion systems in bacteria that are associated with pathogenicity (Dautin, 2021). Type 1 secretion system (T1SS) is defined by ABC transporter, membrane fusion protein and outer membrane protein, which can cooperate to directly transport the matrix of T1SS to the outer membrane of bacteria (Thomas *et al*., 2014). The first T1SS was hemolysin A discovered in uropathogenic *Escherichia coli* causing pyelonephritis (Welch *et al*., 1981), and it had a remarkable effect on the virulence of pathogenic germs (Goebel and Hedgpeth, 1982; Mackman and Holland, 1984).

* Corresponding author: chviolet1984@sina.com
0030-9923/2023/0003-1457 $ 9.00/0

Type 2 secretion system (T2SS) possesses homologous components and conserved mechanisms, which is a macromolecular compound that spans many Gram-negative bacteria and is part of the type IV pili system (Naskar *et al*., 2021). T2SS can be divided into three subunit complexes: An outer membrane protein complex, an inner membrane complex, a pseudo-pilus (Dupuy and Pugsley, 1994). These features of T2SS pledge the performance in bacterial adhesion, biofilm formation, nutrient acquisition and host invasion (Cianciotto and White, 2017), and indirectly boost the generation of antibiotic resistance among bacteria (Santajit and Indrawattana, 2016). Type 3 secretion system (T3SS) was first proposed in 1991, when researchers observed that Yop protein of *Yersinia* was transported into host cells in a non-sec-dependent manner. Subsequently, T3SS of *Salmonella* was discovered in 1998 through negative staining andelectron microscopy (Kubori *et al*., 1998). T3SS was also found in *E. coli* in 2001 (Sekiya *et al*., 2001). Some strain encode the second T3SS, known as ETT2 (Slater *et al*., 2018), which was first discovered in Enterohemorrhagic *E. coli* (EHEC) through genome sequence analysis, and are widely distributed in *E. coli* isolated from humans and animals (Xue *et al*., 2020;

Wang *et al*., 2016). Type 4 secretion system (T4SS) refers to the bacterial secretion system naturally related to the bacterial conjugation mechanism (Lawley *et al*., 2003; Christie *et al*., 2005). As T4SS transport all nucleic acid-protein complexes, T4SS is relatively unique in bacterial secretion system (Juhas *et al*., 2008). Type 5 secretion system (T5SS) is the subject of this article and will not be discussed here. T6SS (Pukatzki *et al*., 2006) was first published in 2006, but does not seemingly involve Sec transport of periplasmic intermediates, commonly found in *Proteus*, including *Vibrio cholerae*, *E. coli*, and *Pseudomonas aeruginosa* (Boyer *et al*., 2009). The virulence effect of T6SS conferred on various pathogens has been confirmed, it could affect the behavior of pathogens in phagocytes (Pukatzki *et al*., 2009; Ma *et al*., 2009), but importantly, conduce to the formation of bacterial biofilm and the killing of other bacteria (Aschtgen *et al*., 2008; Hood *et al*., 2010). Being a relatively rare secretion system that is active in Gram-positive strains, type 7 secretion system (T7SS) was first discovered in the study of *Mycobacterium tuberculosis* (Paulson, 2013), and mainly plays a key role in effector protein secretion of non-pathogenic and pathogenic mycobacteria (such as *Mycobacterium tuberculosis*, the main pathogen of tuberculosis).

In 1989, Normark and his colleagues (Olsén *et al*., 1989; Bhoite *et al*., 2019) reported on the fiber surface structure of *E. coli* suspected to lead to mastitis in dairy cow. Many studies have subsequently revealed a highly regulated biogenic pathway of *Curlybacter* (Dueholm *et al*., 2012), known as type 8 secretion system (T8SS). There are not too many studies on type 9 secretion system-type 11 secretion system (T9SS-T11SS) at present, so I will not elaborate here. In T1SS-T8SS, most of the secretion system are primarily beneficial to Gram-negative bacteria, T1SS, T3SS, T4SS, T6SS as well as T7SS are composed of protein complexes across the inner and outer membrane, and promote secretion through one-step method (Rêgo *et al*., 2010). Additionally, T2SS, T5SS, T8SS and the chaperone-usher (CU) pathways rely on Sec or Tat translocation across the inner membrane. The final translocation is mediated by specific secretion mechanisms, resulting in effector proteins released into the extracellular space or presented on the cell surface (Dautin and Bernstein, 2007).

THE STRUCTURE OF T5SS

There are multifarious secretion systems in negative bacteria, being divided into two categories according to their architectural feature: the first is the large molecular secretion complex that can span the inner and outer membrane of bacteria and the periplasmic space; the latter is the independent secretion mechanism that can pass through the membrane structure of bacteria (Fan *et al*., 2016).

T5SS is conceivable the simplest one among bacterial secretion systems at present owing to most of the secretion systems in T5SS containing only one peptide chain (Fan *et al*., 2016). Moreover, T5SS relies on a polypeptide chain to transport through the outer membrane of bacteria without the consumption of energy such as ATP and cofactors, so T5SS is identified as self-satisfied autotransporter (AT) (Jose *et al*., 1995; Thanassi *et al*., 2005; Drobnak *et al*., 2015; Oberhettinger *et al*., 2015). With the in-depth study of T5SS, although the secretion of ATs depend on manifold substances that we have found, the energy required in the process of T5SS transport has not been specifically described (Thanassi *et al*., 2005). T5SS is mainly divided into five important subtype Va-Ve (Thanassi *et al*., 2005), and a new type Vf was recently discovered (Grijpstra *et al*., 2013). T5SS is a multi-domain protein, despite it comprises plentiful isoforms (Pohlner *et al*., 1987; Henderson *et al*., 1998).

The construction of all T5SS subtypes that we familiar with, were distinguished by the three-dimensional structure of AT, including passenger-protein in T5SS precursor polypeptides and the mutual organization of these domains (Dautin, 2021). The structure of T5SS has been thoroughly studied with obtaining relevant data except for the translocation domain of Vd and Vf (Oomen *et al*., 2004; da Mata Madeira *et al*., 2016; Hage *et al*., 2015). All subtypes share a common domain, which mainly includes three parts (Jose *et al*., 1995; Henderson *et al*., 1998, 2004): (1) Signal peptide sequence: Proteins can be targeted to the inner membrane of the bacteria to deliver them to cyto-periplasm. (2) Passenger domain: Passenger domain endows self-transporters with various functions of effectors. (3) Translocation domain: Translocation domain at C terminal contains a short linker domain; this domain has an α-helical secondary structure and a β domain, where the β domain adapts to the insertion of the β-barrel secondary structure into the outer membrane (Maurer *et al*., 1999; Oliver *et al*., 2003a), and the translocation domain assists in the transport of the passenger domain to the cell surface (Leyton *et al*., 2012). Transport of T5SS through bacterial inner membrane depends on the Sec system (Driessen and Nouwen, 2008), mainly through the cleavable N-terminal signal peptide sequence (Henderson *et al*., 2004). The translocation domain then forms a translocation hole on the outer membrane of the bacterium, through which the passenger domain secretes (Fan *et al*., 2016). The fate of three ATs are vivid emerging in Figure 1. So how do we distinguish between these different subtypes? This principally hinges on the discrepancy of domain composition and transport mechanism. The

structure of each subtype together with related transport mechanism will be introduced below.

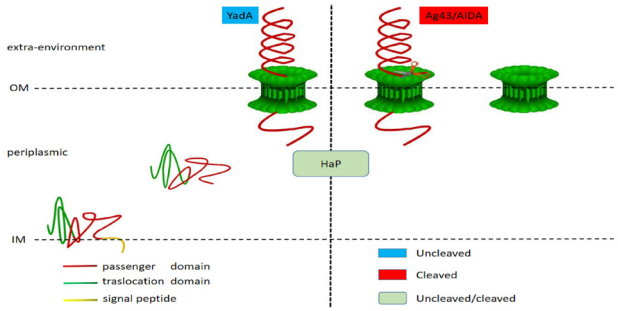

Fig. 1. Schematic overview of type V secretion mechanism. Yellow represents the N-terminal signal peptide, passenger domains are shown in red, while translocation domains in green. There are three different fates to passenger domains. Passenger of adhesin YadA is uncleaved after secretion through translocation domain; instead the Ag43/AIDA are cleaved; For Hap, previous two cases may occur in different situation.

Type Va is the most classic of T5SS, being referred to as the classical AT (Jose *et al*., 1995; Desvaux *et al*., 2004). Focus issue is that how gene encoding the *Neisseria gonorrhoeae* immunoglobulin A1 (IgA1) protease well establish the connection with its extracellular product, (Pohlner *et al*., 1987) explained with a model of T5SS. IgA1 is the first AT of type Va. EstA lipase in *Pseudomonas aeruginosa* and IgA are two ATs that have been researched abundantly in type Va (Henderson *et al*., 2004). The AT contains a β-barrel domain composed of 12 peptide chains, which acts as C-terminal anchor in OM, and is necessary for transport of N-terminal passenger domain to the extracellular environment (Oomen *et al*., 2004). Type Va called canonical AT, resembling the common structure of T5SS described above, possesses single polypeptide chain, mainly including three parts of the domain: Signal peptide sequence, passenger domain and translocation domain. The signal peptide sequence at the N-terminal of the peptide chain can target the target protein to the inner membrane so as to facilitate the transport of the target protein to the periplasm (Henderson *et al*., 1998). The passenger domain, known as the α domain, endows several ATs with different functional factors. Located at the C-terminal, the translocation domain contains a β-pore connecter, which enhances the metastasis of passenger domain to the outer membrane (Maurer *et al*., 1999; Oliver *et al*., 2003a, b; Suzuki *et al*., 1995).

Type Vb is composed of two diverse polypeptide chains encoded by an operon, intituled (given titles) as the two-partner secretion system (TPSS). *Bordetella pertussis* filamentous hemagglutinin (FHA) is a prominent representative (Chevalier *et al*., 2004; Jacob-Dubuisson *et al*., 2013). Jacob-Dubuisson has put forth the term TPSS to draw a number of protein secretion systems that are isogenous and distinct from those previously defined in Gram-negative bacteria (Jacob-Dubuisson *et al*., 2001). It was soon recognized as a branch of T5SS along the AT pathway (Henderson *et al*., 2004). This classification was originally based on some common features between the two traits, but transporters of the Omp85 superfamily were irreplaceable in the secretion of type Vb (Jacob-Dubuisson *et al*., 2013). The TPSS consists of two parts: The translocation domain and the passenger structure are two separating polypeptide chains. The passenger domain or secreted protein is collectively known as TpsA, while the outer membrane protein involved in transport, namely the translocation domain, is TpsB. Like other secretion systems that rely on signal peptides, TPSS primarily uses the Sec mechanism to export TpsA to the periplasmic membrane, subsequently TpsB is inserted into the outer membrane and forms a β-barrel with channel activity to mediate the secretion of TpsA (Henderson *et al*., 2004; Jacob-Dubuisson *et al*., 2004), accompanied by the break of signal peptide in the process of transport.

Compared with other T5SS subtypes, type Vc is probably the most complex subtype. Type Vc is composed of three polypeptide chains, and most of them have the function of bacterial adhesins, thus they are called trimer autotransport adhesins (TAAs) (Linke *et al*., 2006). These proteins are constitutive of three identical polypeptide chains, eventually forming a trimer, which is assembled by a C-terminated 12-chain β-barrel (four β-chains per monomer) and a passenger domain, such as a lollipop structure with a curly helical handle and an N-terminated globular head domain (Linke *et al*., 2006; Hoiczyk *et al*., 2000; Wollmann *et al*., 2006). Unlike classical ATs, the TAAs passenger domains remain covalently sticked to the β-barrel membrane anchors (Wollmann *et al*., 2006), without chopping out after secretion (Wollmann *et al*., 2006). The most intensively studied AT in TAAs is *Yersinin adhesive* A (YadA) (Mühlenkamp *et al*., 2015), both of which are obligate homologous trimer proteins consisting of N-terminal passenger domains and C-terminal translocation units. The N-terminal passenger domain is advantageous to the combination. The constant C-terminal domain is referred as a translocation unit to transport the passenger domain through outer membrane to the extracellular environment.

Type Vd is a 16-chain β-barrel structure homologous to TpsB formed by the combination of an N-terminal passenger domain, a single POTRA (polypeptide transport-related) domain and a C-terminal domain (Salacha *et al*.,

2010). TpsB has two POTRA domains that bind to TpsA substrates in order to stimulate secretion (Leo *et al*., 2012; Brzuszkiewicz *et al*., 2009). Some AT passenger domains in type Va, such as EstA (Brzuszkiewicz *et al*., 2009). have α/β hydrolase folding structures, which are similar to type Vd (da Mata Madeira *et al*., 2016; Emsley *et al*., 1996). Therefore, type Vd is equal to the heterozygotes of Va and Ve subtypes, PlpD in *Pseudomonas aeruginosa* and FplA in *Fusobacterium* are quintessential examples (Salacha *et al*., 2010; Casasanta *et al*., 2017). The overall structure of type Vd is incredibly similar to type Va, except that these domains are connected by an additional periplasmic domain homologous to the periplasmic domain of type Vb translocation pore (Fan *et al*., 2016).

Structure of Ve is quite peculiar. The C-terminal is a slender passenger domain composed of multiple independent immunoglobulin-like domains (Oberhettinger *et al*., 2012, 2015), while N-terminal translocation domain is a 12-chain β-barrel formed in the extracellular membrane (Hamburger *et al*., 1999; Fairman *et al*., 2012; Leo *et al*., 2015b). The passenger domain of Ve is immunoglobulin-like or hemagglutinin like, which is absence in other T5SS, but widely present in Gram-positive bacteria (Bateman *et al*., 1996; Bodelón *et al*., 2013). Type Ve secretion systems contain a periplasmic domain at the N-terminus of the polypeptide chain, which may play a certain auxiliary role in the dimerization and interaction of peptidoglycan, possibly anchoring it and promoting the interaction between receptors during host invasion (Leo *et al*., 2015a).

Type Vf is the most controversial subtypes initially discovered in recent years, BapA and SabA are typical representative, which seem to be unique to *H. pylori*, have a surface-exposed domain inserted into the N-terminal region between the first and second β chains of the 8-chain β-barrel domain, and contain few additional passenger domains at either the ends off peptide chain. Therefore, passenger domain is actually an extended ring of the β-barrel domain, which is smaller than any AT (Coppens *et al*., 2018). Although BapA and related proteins are considered as ATs, their topology is quite dissimilar to other types of ATs (Meuskens *et al*., 2019). The topological structure of Hop C-terminal domain is different from others. For Hop, multiple sequence alignment and transmembrane β-chain prediction indicate that there are 7 β-chains in the C-terminal domain (Alm *et al*., 2000), but general AT structures share a common 12-chain β-barrel structure, monomer ATs are made up of consecutive 12 β-chains, and trimer ATs are composed of three 4-chain β slices in a composite β-barrel structure (Oomen *et al*., 2004; Leyton *et al*., 2012; Meng *et al*., 2006). Therefore, it is doubtful whether type Vf should be confirmed as a member of T5SS, and further study of their secretion mechanisms is needed to determine if these proteins actually transport in a manner similar to other ATs.The structure of T5SS subtypes previously mentioned are exhibited in Figure 2.

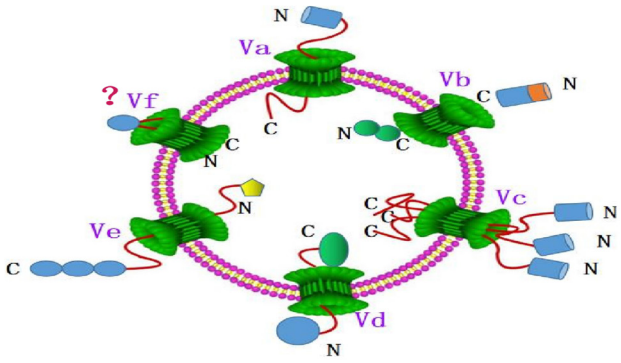

Fig. 2. Schematic V secretion system subclasses. B-barrels and POTRA domains are shown in green, linkers and TPS domains in red, and passenger domains in blue. The periplasmic extension of type Ve proteins is in golden. The positions of the N- and C-termini are indicated. Type Vf is not fully established as part of T5SS.

FUNCTION OF T5SS

Most of the ATs by T5SS are the expression products of bacterial virulence factors, contributing actively to the pathogenic mechanism of bacteria. T5SS is mainly found in Gram-negative bacteria and plays a synergistic role in bacterial pathogenicity, such as bacterial invasion (Capecchi *et al*., 2005), serum resistance (Attia *et al*., 2005), adhesion (Bullard *et al*., 2005), biofilm formation (Valle *et al*., 2008). Adhesion is the first important step for a large proportion of intestinal microorganisms to colonize and persist in the intestinal tract of the host (Pizarro-Cerdá and Cossart, 2006), which lays a foundation for the subsequent formation of bacterial biofilm and the operation of other pathogenic effects. Adhesin plays an important role in the pathogenicity of pathogens. Adhesin-like ATs exist in all subtypes of T5SS, such as AIDA (Laarmann and Schmidt, 2003) and EhaA (Charbonneau *et al*., 2006) in type Va; Adhesion of *Bordetella pertussis* and FHA of in type Vb (Aricò *et al*., 1993; Serra *et al*., 2011); YadA of *Yersinia* in type Vc (Tertti *et al*., 1992) and *E. coli* tight adhesion in type Ve (Kenny *et al*., 1997).

In the evolution process of continuous struggle between bacteria and host, the strategies of bacterial adhesion to host cells have undergone great changes. Autotransporter proteins promote bacterial adhesion to host cells by forming biofilm (Hall-Stoodley *et al*., 2004; Yan and Bassler, 2019), non-covalent binding (Sauer *et al*., 2016) or direct covalent binding (Walden *et al*., 2015). The

formation of biofilm can significantly enhance the virulence of bacteria and lead to host asthenia, for instance, the ATSs YrInv and YrIlm of type Ve in *Yersinia ruckeri* (Wrobel *et al*., 2020). Dirk linke (Wrobel *et al*., 2020) confirmed through that the self-transporters YrInv and YrIlm colonized on the surface of host cells and participated in the formation of biofilm. The well-understood AT Ag43 significantly strengthens the formation of bacterial biofilm, mainly due to the eminent cell aggregation properties of Ag43, and Ag43 is a unique self-identifying adhesin, on account of all receptor recognition and receptor targets are provided by the same polypeptide (Kjaergaard *et al*., 2000; Hasman *et al*., 1999). These traits are crucial reasons to promote the formation of bacterial biofilm.

Why biofilm formation is beneficial to the pathogenicity of bacteria? Because it can make bacteria attach to the host cell more stable than the free state, and it is not easy to be washed away by the flowing liquid. Meanwhile, it can resist the bactericidal effect of many antibiotics, which is a crucial reason for bacteria to develop drug resistance (Costerton *et al*., 1999). Overall, the AT secreted by T5SS indirectly enhances the virulence and pathogenicity of bacteria by promoting the formation of biofilm.

AT can participate in the pathogenic activities of bacteria through the functions of lipase esterase protease and other enzymes (da Mata Madeira *et al*., 2016; Casasanta *et al*., 2017; Ocampo *et al*., 2021). The representative AT EstA with lipase domain plays multiple roles in the pathogenic process of bacteria, primarily involving in the formation of bacterial biofilm (Davey *et al*., 2003; Tielen *et al*., 2010), motility of bacteria (Wilhelm *et al*., 2007) and lipid hydrolysis of bacteria (Carinato *et al*. 1998), as well as cell signal transduction (Riedel *et al*., 2003). Protease ATs can be classified into three categories: Enterobacteriaceae serine protease ATs (SPATEs); Non-SPATEs class ATs, SPATEs like AT. EspP, the model of SPATEs (Roman-Hernandez *et al*., 2014), is a vital virulence factor of EHEC, playing a role in cytotoxic adhesion and biofilm formation. Generally detected in *Diarrheagenic E. coli* (DEC), EspP can cleave molecules of the complement system, exacerbating the severity of hemolytic uremic syndrome caused by EHEC (Orth *et al*., 2010). SPATEs like ATs are represented by IgA protease (Diebel *et al*., 2004), while Non-SPATEs ATs, represented by NalP (Arenas *et al*., 2013), are involved in the pathogenic effect of bacteria on the host.

Pathogens that invade tissues and spread systematically before encountering innate immune defenses of complement is based on the ability to evade the various killing mechanisms initiated by the complement cascade. These mechanisms include cleavage of membrane-attacking complexes formed by the outer membrane of Gram-negative pathogens, opsonization by complement components, and recruitment of phagocytes through production of allertoxins (Ehrengruber *et al*., 1994; Klos *et al*., 2009). Some bacteria can survive and proliferate in serum, which is related to T5SS. Numerous ATs of T5SS destroy complement molecules in serum to stimulate the proliferation of bacteria. YadA (Schindler *et al*., 2012) adhesin of *Yersinia colitis* and Vag8 (Marr *et al*., 2011) adhesin of *Bordetella pertussis*, for example, inhibit the cascade reaction of complement by binding with complement protein to avoid bacterial lysis. Therefore, T5SS is of tremendous significance in the serum resistance of bacteria.

T5SS is versatile, in addition to the above functions, including participation in contact-dependent growth inhibition (CDI) (Guérin *et al*., 2017), bacterial aggregation (Trunk *et al*., 2018), bacterial invasion, cytolysis (Reboud *et al*., 2017) and immune intrusion (Schindler *et al*., 2012), etc. However, due to the lack of in-depth understanding of some pathogenic mechanisms, urgent studies are needed to clarify its role in pathopoiesia.

THE POTENTIAL APPLICATION OF T5SS

England's Chief Medical Officer Dame Sally Davies had said that the issue of continuous antibiotics like climate action, affecting daily lives in no small way (Wiersinga *et al*., 2020). The rapidly expanding AT family is the largest of Gram-negative bacteria toxicity protein family, contains more than 700 different virulence factor, associated with many diseases, such as meningitis, septicemia perineum pericarditis Otitis media, sinusitis, pneumonia, diarrhea, septicemia, and peptic ulcer (Henderson and Nataro, 2001). As a result, developing treatments and strategies for drug-resistant superbugs is imperative and urgent. Now it is clear that the adhesion and the adhesion and aggregation of bacteria is the first and pivotal step on the bacteria invade the organism and induce disease (Fux *et al*., 2005). T5SS pathogenic mechanism in gram-negative bacteria play a vital role in the first step (Dunne, 2002), so utilizing the function of T5SS in bacterial pathogenesis mechanism to research related biological products, for reducing the morbidity and mortality caused by Gram-negative bacteria are of real significance. Next, we will discuss the specific strategies and the prospects for future research of taking advantage of T5SS to prevent Gram-negative bacterial diseases.

Evidences from previous studies reveal that encoding sequence and function of T5SS subtypes are increasingly diversified while their secretion mechanisms conforming to the same path, so we can take advantage of this trait

inhibiting bacterial secretion system running. *Haemophilus influenzae* outer membrane protein D15, a homologue of Omp85, has been shown to be highly immunogenic in animal models such as mice, guinea pigs and rabbits (Loosmore *et al*., 1997). FhaC structure is an Omp85-like homologue in the TPS secretion system, from which a prospective drug design strategy can be derived (Clantin *et al*., 2007). Currently, a few major recombinant vaccines with ATs have been applied into clinical, for instance, the acellular vaccines composed of FHA and Pertactin (Whelan *et al*., 2020).

Ag43 induces interspecific cell-to-cell contact and promotes the formation of biofilm of multiple species. The data (Kjaergaard *et al*., 2000) indicates that the multi-functional molecular tool is used in reasonable design for multi-species biofilm. More specifically, this new technique provides the opportunity to design multi-species collaborations, which are necessary for bacterial collaboration, such as waste treatment and pollutant degradation, affording new ideas for ecological applications of bacterial T5SS. In addition, bacterial biofilm formation has an effect on the development of bacterial drug resistance, reducing or eliminating the ability of bacteria biofilm formation can inhibit the generation of the antimicrobial resistance. Therefore, interfering with T5SS transporter can achieve inhibiting the formation of bacterial biofilm, which offers a new way for the antibiotic treatment of bacterial disease.

THE PROBLEMS TO BE SOLVED AT PRESENT

Some studies have confirmed the initial model of T5SS, but it has been doubtful because several important questions about the structure and function of this pathway remain to be resolved: (1) what energy drives secretion in the passenger domain? (2) Whether there are auxiliary factors involved in the process of periplasmic transport and assembly to the outer membrane? (3) Is the secretion mechanism of AT of each subtype consistent? (4) What role does POTRA domain play in type Vc? (5) What are the factors involved in the course of cell surface protein generation? Are intrinsic factors, such as the self-chaperone domain, required for AT secretion? Existing research strategies in some laboratories will no doubt resolve these dilemmas in the coming years, providing a fuller understanding of secretion mechanisms and perhaps revealing new variations on the subject of T5SS. For example, it was previously thought that the trimer auxilin in T3SS secretion coordinates the secretion of three polypeptide chains (Grin *et al*., 2014), the latest studies prefer the simultaneous secretion of three passenger domains in the Vc subtype (Chauhan *et al*., 2019). Meanwhile, some scholars (Whelan *et al*., 2020) suggested that T5SS promotes the rearrangement of bacterial cytoskeleton in coordination with T3SS, then protecting bacteria and mediating the pathogenic effect of bacteria on organs.We highly look forward to the brilliant breakthrough in clinical application or mechanism when the unsovled issue about T5ss would be clarified.

ACKNOWLEDGEMENT

We would like to thank Chun Qing Wu for revision of this paper.

Funding

The study was supported by the Wenzhou cooperation group for breeding new poultry varieties project (No. 2019ZX005), Wenzhou Engineering Research Center of Pet (WP04), Wenzhou Basic Scientific Research Project(N2019007) and Sub-project of cooperation Group of Breeding new varieties of poultry in Wenzhou(2019ZX005-5).

Statement of conflict of interest

The authors have declared no conflict of interest.

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