Review Article

BACTERIAL DELIVERY SYSTEMS FOR IMMUNOGENIC PROTEINS: CURRENT ADVANCES, LIMITATIONS AND CHALLENGES

Km. Himani

Department of Veterinary Clinical Complex
Arawali Veterinary College, Sikar
Rajasthan University of Veterinary and Animal Sciences, Bikaner
Rajasthan – 332 403

ABSTRACT

Bacterial platforms are emerging as versatile systems for delivering heterologous antigens and therapeutic proteins, driven by advances in microbial engineering and insights into host–pathogen interactions. This review summarizes recent peer-reviewed literature on bacterial delivery platforms, focusing on their mechanisms, immunological properties, applications, and limitations. Major approaches including live attenuated bacteria, secretion systems, surface display technologies, spores, outer membrane vesicles (OMVs), and bacterial ghosts (BGs) enable targeted intracellular or mucosal delivery and can elicit strong humoral, cellular, and innate immune responses. Live vectors naturally target antigen-presenting cells, while secretion systems such as the Type III secretion system (T3SS) allow precise cytosolic delivery. Spores provide stable, low-cost vehicles, and OMVs and BGs offer non-replicating particulate platforms with intrinsic adjuvanticity. Despite their potential, each system faces limitations, including low immunogenicity (spores), LPS toxicity and variability (OMVs, BGs), biosafety concerns (live vectors), and size or folding constraints (secretion and surface-display systems). Advancements in synthetic biology, immunomodulation, and scalable manufacturing are needed to overcome these barriers. Collectively, bacterial delivery platforms hold significant promise for next-generation vaccines and therapeutic protein delivery.

Keywords: Bacterial delivery systems, antigen delivery, outer membrane vesicles, bacterial ghosts, vaccine engineering.

Received : 26.05.2026 Revised : 21.08.2026 Accepted : 25.08.2026

INTRODUCTION

Over the past four decades, advances in bacterial engineering, molecular biology, and our understanding of pathogenic bacterial lifestyles have significantly accelerated the rational design of bacterial vectors for in vivo delivery of heterologous antigens into antigen-presenting cells (APCs). The increasing incidence of infectious diseases has heightened the demand for efficient vaccines, biologically derived carriers, and targeted delivery systems. Intracellular delivery of proteins, including antigens, transcription factors, signaling molecules, and gene-editing enzymes, plays a pivotal

[1]Assistant Professor
*Corresponding Author Email : himanidhiman918@gmail.com

role in biomedical research, encompassing cellular and molecular biology, immunology, and medical genetics (Woltjen *et al.,* 2009). Transient protein delivery offers a safe alternative to gene-based approaches, mitigating concerns about genome disruption or incomplete silencing of exogenous genes. However, the delivery of proteins into mammalian cells remains challenging due to the limited capacity of proteins to cross cell membranes.

Bacterial vectors have emerged as versatile platforms for protein and antigen delivery, with potential applications in vaccine development and immunotherapy. Advances in genetic engineering have enabled the construction of recombinant microorganisms capable of expressing heterologous proteins in distinct cellular compartments, thereby enhancing their antigenic potential for vaccines targeting viruses, bacteria, and parasites.

In parallel, bacterial secretion systems and naturally secreted nanostructures, such as outer membrane vesicles (OMVs) and bacterial ghosts (BGs), have opened new avenues for targeted delivery. Gram-negative pathogens utilize type III secretion systems (T3SS) to translocate effector proteins into host cells, a mechanism evolutionarily related to the flagellar apparatus (Cornelis and Wolfwatz, 1997). Understanding these mechanisms has provided insights into designing bacterial platforms for antigen and therapeutic delivery. Synthetic nanoparticles offer alternative delivery approaches, yet they often lack the complex intercellular interaction motifs inherent to natural bacterial systems (Hu *et al.,* 2015).

Advances in synthetic biology and genetic engineering have further expanded bacterial platforms from passive antigen carriers to programmable delivery systems. Engineered bacteria can be designed for controlled expression and secretion of therapeutic proteins, and localized payload release, thereby improving delivery specificity while potentially reducing systemic toxicity (Dey and Sankaran, 2024). Bacteria-based systems are also being explored beyond vaccination for targeted delivery of biologics and anticancer therapeutics, with engineered secretion circuits and surface-targeting modules enabling site-specific therapeutic activity (Shahid *et al.,* 2024). However, challenges related to biosafety, containment, manufacturing consistency, host–microbe interactions, and clinical translation remain important considerations for the development of next-generation bacterial delivery platforms.

This review provides a comprehensive overview of current bacterial-based delivery systems, including live bacteria, bacterial surface proteins, spores, OMVs, and BGs.The review aims to compare the delivery mechanisms, evaluate their applications in vaccine and therapeutic protein delivery, critically assess their advantages and limitations, and discuss emerging strategies and future directions for improving their safety, efficacy, and translational potential.

MATERIALS AND METHODS

Data Description

The data for this study were sourced from secondary databases, specifically focused on bacterial delivery platforms, including live vectors, secretion systems, surface display, spores, OMVs, and bacterial ghosts, were selected based on their mechanisms, applications, immunological properties, advantages, and limitations. The selected literature was qualitatively synthesized to provide a comparative assessment of these delivery platforms.

Live Bacteria as Delivery Vehicles

The use of live bacterial cells as vectors for recombinant antigen delivery has gained significant attention over the past two decades. The innate immune-stimulating properties of bacterial pathogens—mediated by pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharides (LPS) in Gram-negative bacteria and lipoteichoic acids in Gram-positive bacteria—activate pattern recognition receptors (PRRs) on host cells. This triggers inflammatory cytokine production and antimicrobial gene expression, thereby bridging innate and adaptive immune responses (Janeway and Medzhitov, 2002). Live bacterial vectors naturally target APCs, facilitating antigen presentation and robust immune activation. For example, *Salmonella enterica* invades intestinal M cells and macrophages, enabling heterologous antigen exposure to the host immune system (Jones *et al.,* 1995). Dendritic cells also capture these bacteria in the lamina propria, further enhancing immune priming (Rescigno *et al.,* 2001). The localization of antigens within the bacterial vector significantly influences the magnitude and type of immune response. Surface-expressed antigens, often fused to bacterial surface proteins such as Lpp-OmpA, FliC, or OprF, primarily elicit humoral responses, whereas secreted antigens can enhance cellular immunity (Kaufmann and Hess, 1999; Kotton and Hohmann, 2004). Live bacterial vaccines are being actively developed, with companies such as Advaxis and Aduro Biotech advancing Listeria-based vectors into clinical trials targeting HPV and tumor-associated antigens, demonstrating both safety and immunogenicity in humans (Le Gouëllec *et al.,* 2012). Mucosal delivery of live bacterial vaccines offers the additional advantage of inducing local and systemic immunity, reducing side effects and lowering production costs (Cortes-Perez *et al.,* 2007). Synthetic biology have enabled precise attenuation, metabolic engineering, and genetic control of bacterial vectors, improving their safety and therapeutic potential. Engineered Salmonella and other live bacterial platforms are increasingly being investigated for vaccination, targeted antigen delivery and cancer therapy, although biosafety, containment, and translational challenges remain (Bansal *et al.,* 2024; Kwon *et al.*, 2024).

Live bacterial vehicles with various modes of delivery: (1) Intracellular expression, (2) secretion, (3)membrane- and cell wall display, (4) pilus-mediated display (5) heterologous display

Bacterial Secretion Systems as Protein Delivery Tools

Bacteria have evolved sophisticated secretion systems to transport proteins across their cell envelopes into the extracellular environment or directly into host cells. Among these, Type III, Type IV and Type VI secretion systems (T3SS, T4SS and T6SS) are particularly well-characterized for their roles in pathogenesis and are increasingly repurposed as precise tools for intracellular delivery of immunogenic proteins (Mori and Ito, 2001). The T3SS, often described as a "molecular syringe" or injectisome, is extensively studied in Gram-negative pathogens such as Salmonella, Shigella, and Yersinia. This system enables direct translocation of effector proteins into the host cytosol, bypassing extracellular degradation and allowing temporal and spatial control of antigen presentation—a critical feature for vaccines targeting intracellular pathogens (Cornelis and Wolfwatz, 1997; Toussaint *et al.,* 2013).

Structure and Mechanism of T3SS

The T3SS injectisome is a multimeric protein complex comprising over 20 proteins, forming a needle-like apparatus spanning the bacterial envelope (Buttner, 2012). The basal structure, anchored in inner and outer bacterial membranes, houses an ATPase that powers effector translocation. Recent structural studies using cryo-electron microscopy (cryo-EM) and cryo-electron tomography (cryo-ET) have provided detailed insights into the architecture and dynamic organization of the injectisome, including its membrane rings, export apparatus, cytoplasmic sorting platform, and ATPase complex (Chen and Goldberg, 2023; Worrall *et al.,* 2023).The extracellular needle, with a narrow internal channel of approximately 2–3 nm, provides a continuous conduit through which secretion substrates are transported in an unfolded or partially unfolded state (Andrade *et al.,* 2007; Chen and Goldberg, 2023).The needle tip or translocon inserts into the eukaryotic membrane, allowing effectors to enter host cells, where host folding machinery refolds them into active conformations. Effector proteins can manipulate host cell processes, promote bacterial attachment, evade immune responses, or induce apoptosis. Functional T3SS activity is therefore an important determinant of virulence, and disruption of T3SS components generally results in reduced pathogenicity (Kimura *et al.,* 2011; Pais *et al.*, 2023).

Engineering Bacteria for Heterologous Protein Delivery

Several pathogenic bacteria naturally encode T3SS as a virulence factor, including Salmonella, Shigella, enteropathogenic *E. coli* (EPEC), Yersinia, *Pseudomonas aeruginosa*, and *Xanthomonas campestris*. To repurpose T3SS for protein delivery, these pathogens are first attenuated to reduce cytotoxicity while maintaining secretion capacity.

Salmonella spp. have been engineered as vectors for heterologous antigens, eliciting robust humoral, mucosal, and cellular immunity upon oral

administration. Attenuation strategies include deletions of virulence genes (ΔmsbB, ΔsptP, ΔphoP/phoQ) and metabolic auxotrophies (Δasd, ΔpurI/purD, ΔaroA) to enable self-limiting growth in vivo while preserving immunogenicity (Panthel *et al.,* 2008; Frahm *et al.,* 2015; Juárez-Rodríguez *et al.,* 2012).

Yersinia spp. encode T3SS on the virulence plasmid pYV, delivering Yersinia outer proteins (Yops) to modulate host intracellular processes. Attenuated strains, such as ΔYopHOPEMT combined with asd deletion, maintain the ability to translocate proteins without cytotoxicity, providing a platform for heterologous antigen delivery (Trülzsch *et al.*, 2003; Ittig *et al.*, 2015).

Pseudomonas aeruginosa T3SS delivers exotoxins (ExoS, ExoT, ExoY, ExoU) to evade host defenses. Its ~40-gene cluster, organized in five operons, can be exploited for protein delivery following attenuation and modulation of secretion pathways.

Strategies for Heterologous Protein Secretion

Two principal approaches are employed to target proteins via T3SS: secretion tagging and whole-protein fusions. Secretion Tags: Effector proteins often contain N-terminal sequences recognized by the T3SS sorting platform. Cytosolic chaperones bind these tags to stabilize effectors, prevent premature folding, and maintain the linear conformation necessary for translocation (Akeda and Galan, 2005). By appending these tags to heterologous proteins, they can be efficiently secreted. Translocation efficiency varies among tags, allowing hierarchical delivery of multiple effectors (Munera *et al.*, 2010). For instance, Salmonella minimal translocation sequences from SspH2 have been optimized to deliver adenylate cyclase reporter fusions into host cells (Miao and Miller, 2000).

Whole-Protein Fusions: Fusing heterologous proteins to native effectors addresses folding constraints and ensures translocation through the T3SS needle. Chaperone-binding domains stabilize the fusion, facilitating efficient delivery. The necessity of chaperone pairing is exemplified by SseB-CyaA fusions in Salmonella, where deletion of the SseA chaperone abolished secretion (Zurawski and Stein, 2003).

Bacterial Surface Proteins as Delivery Vehicles

Bacterial surface display systems exploit naturally expressed surface proteins for antigen presentation. These proteins can trigger host immune recognition and serve as platforms for engineered immunotherapeutics.

S-layer Proteins: S-layer proteins form crystalline arrays on bacterial surfaces and exhibit strong adjuvant properties. They have been employed as carriers for allergens and antigens, demonstrating enhanced T-cell responses and Th1 polarization in experimental models (Sleytr *et al.*, 2014; Jahn-Schmid *et al.*, 1997). Recombinant fusion strategies have further improved

S-layer-based delivery, enabling endotoxin-free antigen presentation in non-pathogenic hosts such as Bacillus subtilis (Ilk *et al.,* 2011).

Albumin Binding Protein: Fusion of antigens to streptococcal albumin-binding proteins has enabled the presentation of IgE mimotopes in a monovalent form, eliciting protective IgG responses without triggering allergic reactions, highlighting the safety and versatility of surface protein-based carriers (Ganglberger *et al.*, 2001).

Bacterial Spores as Delivery Vehicles

Bacterial spores, particularly from *Bacillus subtilis,* offer a durable, safe and cost-effective platform for antigen delivery (Duc *et al.*, 2003). Recombinant antigens can be displayed on the spore surface or expressed intracellularly using appropriate promoters, facilitating antigen protection and delivery to mucosal immune sites (Paccez *et al.,* 2007; Saggese *et al.*, 2023). Surface-expressed antigens favor Th1 responses, whereas cytoplasmic expression during germination promotes Th2 responses (Uyen *et al.,* 2007). Recent studies demonstrate that *B. subtilis* spores possess intrinsic immunostimulatory properties and can enhance innate and adaptive immune responses, supporting their application as oral and intranasal vaccine carriers (Yuan *et al.,* 2024). Spore-based vaccines have demonstrated protective efficacy in parasite challenge models, underscoring their potential as versatile immunization tools.

Outer Membrane Vesicles (OMVs) as Delivery Vehicles

OMVs are naturally secreted nanoscale vesicles from Gram-negative bacteria, comprising LPS, proteins, phospholipids, and periplasmic content (Liu *et al.*, 2016). OMV biogenesis is often stress-induced and enables the efficient packaging of bacterial components. Their multifunctional composition allows simultaneous delivery of antigens and intrinsic adjuvanticity, promoting both innate and adaptive immune responses (Underhill and Goodridge, 2012; Toyofuku *et al.*, 2023). OMVs interact with various immune cells, including dendritic cells, macrophages, neutrophils, and eosinophils, inducing proinflammatory cytokines and promoting T- and B-cell activation (Jager *et al.*, 2014; Ko *et al*., 2015). They also modulate non-immune cells, enhancing endothelial adhesion molecule expression and leukocyte recruitment (Kim *et al.,* 2013). These attributes render OMVs promising candidates for vaccine delivery and immunotherapy, though challenges such as endotoxin toxicity, scalable production, and standardized characterization remain (Hu *et al*., 2015; Toyofuku *et al*., 2023).

Bacterial Ghosts (BGs) as Delivery Vehicles

Bacterial ghosts are empty envelopes of Gram-negative bacteria produced through controlled expression of bacteriophage phiX174 lysis gene E (Henrich *et al.,* 1982). The E protein forms transmembrane

tunnels that expel cytoplasmic content while preserving the cell envelope, including surface antigens and pathogen-associated molecular patterns. BGs can be loaded with heterologous antigens in the lumen, periplasm, or on the surface, making them highly versatile delivery platforms (Mayr *et al.,* 2005). BGs are efficiently recognized by APCs and induce robust humoral and cellular immunity, while the LPS content contributes adjuvant activity with minimal toxicity (Jawale and Lee, 2014; Cai *et al.,* 2015). Fusion strategies using anchor sequences or secretion signals allow targeted localization of antigens within the BG, enhancing immune responses. Engineered BGs have demonstrated efficacy in antigen delivery through DNA-vaccine and surface-antigen display approaches (Yu *et al.*, 2022). Lipidated antigen-engineered *E. coli* ghosts elicited strong humoral and Th1-biased cellular responses and enhanced clearance of *Chlamydia abortus* infection in mice, highlighting their potential as adjuvant-free veterinary vaccine platforms (Zhang *et al.,* 2024). BG-based vaccines have demonstrated protective efficacy in models of *Chlamydia trachomatis* genital infection, suggesting their potential for mucosal and systemic immunization (Chakameh *et al.,* 2004).

Comparative Overview of Bacterial Delivery Platforms

Bacterial systems have evolved diverse strategies to deliver antigens, therapeutic proteins, and nucleic acids. Table 1 summarizes the key bacterial delivery vehicles discussed in this review, highlighting their mechanisms, advantages, limitations, and applications.

Limitations and Challenges of Bacterial Delivery Platforms

Despite the significant progress in engineering bacterial systems as antigen and protein delivery vehicles, each platform faces intrinsic limitations that hinder translational application. Bacterial spores, such as those from *Bacillus subtilis*, exhibit low intrinsic immunogenicity, often requiring potent adjuvants to achieve strong responses. Their spore coat architecture restricts the size and complexity of antigens that can be displayed, and overexpression may compromise sporulation efficiency. Oral delivery further suffers from rapid gastrointestinal transit and limited mucosal retention, while strain-to-strain variability in coat composition complicates reproducibility and regulatory standardization. Likewise, outer membrane vesicles (OMVs) are challenged by the inherent toxicity of their lipopolysaccharide (LPS) content, which, although adjuvanting, risks excessive inflammation. OMV production remains difficult to scale given the reliance on ultracentrifugation and the heterogeneity of naturally packaged cargo, resulting in batch variability. Furthermore, antigen-loading strategies are still imprecise, and standardized analytical methods for particle quantification, cargo loading, and pharmacokinetics remain underdeveloped. Bacterial ghosts (BGs) also present hurdles, including complex production processes requiring tightly controlled lysis conditions and often yielding low quantities. While residual endotoxin levels are reduced, they

still pose concerns for clinical use. Antigen loading is constrained by the need to fuse proteins to membrane anchors or periplasmic targeting signals, limiting the size and structural complexity of deliverable cargo. Additionally, BG stability can be sensitive to storage conditions, and the reproducible large-scale manufacturing of structurally intact ghosts remains technically demanding. Live attenuated bacteria, although potent inducers of mucosal and systemic immunity, present inherent biosafety challenges. Risks include reversion to virulence, horizontal gene transfer of resistance markers, and environmental transmission. Pre-existing immunity against the vector strain may diminish efficacy, and maintaining stable expression of heterologous antigens imposes high metabolic burden, often leading to genetic instability and attenuation of antigen expression. Regulatory approval for live vectors also requires extensive safety evaluation, particularly for immunocompromised populations. Complexity is further magnified in bacterial secretion systems such as T3SS, T4SS and T6SS. These systems require attenuation of cytotoxic parent strains to ensure safety, which can inadvertently limit secretion efficiency. Structural constraints of secretion machinery—particularly the narrow T3SS needle—necessitate translocation of proteins in an unfolded state, resulting in poor delivery efficiency for large or highly stable proteins. Expression and secretion of heterologous cargo are often inconsistent due to mRNA secondary structures, chaperone competition, and hierarchical secretion order. Engineering custom secretion tags, effector fusions, or chaperone interactions adds additional layers of complexity, and host immune responses against bacterial machinery may limit repeated dosing. Finally, surface display systems are constrained by antigen size and folding limitations, as large or structurally complex proteins may destabilize surface anchoring or impair bacterial viability. Surface-exposed antigens are susceptible to proteolysis and environmental degradation, and tuning antigen orientation or density to achieve predictable immune polarization remains challenging. High expression levels impose metabolic stress on host bacteria, while species-specific differences in cell surface architecture necessitate bespoke engineering strategies for each antigen–bacterium combination.

CONCLUSION

Bacterial platforms offer versatile and innovative strategies for the delivery of vaccines, therapeutic proteins, and other biologics. Systems such as bacterial spores, outer membrane vesicles (OMVs), bacterial ghosts, live attenuated bacteria, bacterial secretion systems, and surface display approaches each provide unique advantages, including robust stability, intrinsic adjuvanticity, and the ability to target antigens to specific cellular compartments. These features make them attractive alternatives to conventional delivery systems and highlight their potential in eliciting potent humoral, mucosal, and cellular immune responses. Despite these advantages, significant challenges remain that limit their broad clinical translation. Bacterial spores face low intrinsic

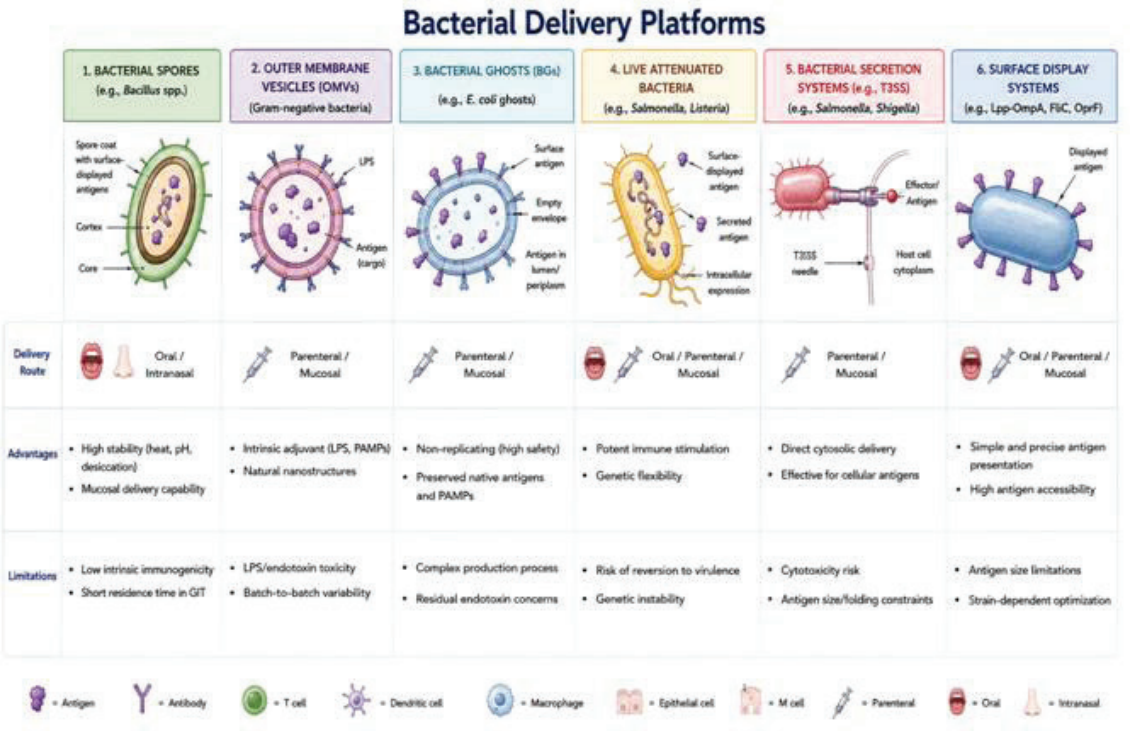
immunogenicity and short gastrointestinal residence time, while OMVs are constrained by LPS toxicity, batch variability, and cargo heterogeneity. Bacterial ghosts, though safe and immunogenic, suffer from complex production processes, low yield, and residual endotoxin concerns. Live attenuated bacteria present risks of reversion to virulence, horizontal gene transfer, and instability of recombinant antigens, whereas bacterial secretion systems, such as T3SS, require careful engineering to overcome cytotoxicity, folding constraints, and variable translocation efficiencies. Surface display systems are limited by antigen size, stability, and strain-specific optimization requirements. Collectively, these challenges underscore the need for continued refinement in strain engineering, antigen design, delivery optimization, and scalable manufacturing processes. Future research should focus on integrating synthetic biology, protein engineering, and immunomodulatory strategies to enhance antigen stability, improve targeted delivery, and minimize host toxicity. Combining multiple bacterial delivery platforms or incorporating hybrid systems may further enhance efficacy and safety profiles. With these advancements, bacterial-based delivery systems hold the promise of transforming vaccinology and therapeutic protein administration, offering precise, safe, and highly adaptable tools for next-generation immunotherapies.

Future research should prioritize the development of genetically controllable and precisely attenuated and genetically controllable bacterial platforms, standardized manufacturing, and rigorous evaluation of safety and immunogenicity. Integration of multi-omics, synthetic biology, and AI-assisted protein design may enable rational optimization of antigen–carrier systems and targeted delivery. Scalable manufacturing, regulatory harmonization, and robust translational studies remain essential for advancing bacterial delivery platforms toward clinical application.

Table 1. Comparative Overview of Bacterial Delivery Systems

Delivery System	Mechanism	Advantages	Limitations/ Challenges	Application
Bacterial Spores (e.g., *Bacillus subtilis*)	Dormant spores expressing antigen on surface or within spore	Heat-resistant, stable in harsh conditions, GRAS status, low production cost, probiotic effect	Low immunogenicity, short GI residence, limited antigen expression	Oral/nasal vaccines, mucosal immunity induction

Outer Membrane Vesicles (OMVs)	Naturally secreted nanoscale vesicles from Gram-negative bacteria, carrying LPS, proteins, periplasmic components	Self-adjuvanting (LPS),multi-antigen presentation, interaction with APCs, can elicit both humoral & cellular immunity	LPS toxicity, scalable purification challenges, heterogeneity, PK/PD characterization needed	Bacterial vaccines, antigen delivery, therapeutic delivery
Bacterial Ghosts (BGs)	Empty Gram-negative envelopes generated by controlled lysis (gene E); antigens can be loaded internally or expressed on surface/periplasm	Safe, non-replicating, particulate nature facilitates APC uptake, contains TLR agonists, flexible antigen loading	Complex production, LPS toxicity, low yield efficiency	Vaccine delivery, viral & bacterial antigen presentation, adjuvant applications
Live Attenuated Bacteria	Replication-competent bacteria engineered to express heterologous antigens	Strong immunogenicity, can induce systemic, mucosal, and cellular immunity, versatile antigen delivery	Risk of reversion to virulence, horizontal gene transfer, pre-existing immunity, stability issues	Traditional and recombinant vaccines, intracellular pathogen models
Bacterial Secretion Systems (T3SS, T4SS, T6SS)	Injectisome-mediated delivery of linearized effector proteins into host cytosol	Direct intracellular delivery, precise temporal/spatial control, potent antigen-specific T cell responses, compatible with live attenuated or non-replicating vectors	Cytotoxicity of parent strain, folding/size constraints of cargo, variable expression and translocation efficiency	Intracellular antigen delivery, vaccine developmen, antiviral activity
Bacterial Surface Display Systems	Heterologous antigens anchored on bacterial surface via fusion proteins (OmpA, pili, spore coat proteins)	Easy APC recognition, can display multiple antigens, simple genetic engineering	Limited antigen size, potential interference with bacterial fitness, low antigen stability	Oral vaccines, subunit vaccines, mucosal immunity induction



Fig,1. Bacterial Delivery Platforms

REFERENCES

Akeda, Y. and Galán, J.E. (2005). Chaperone release and unfolding of substrates in type III secretion. *Nature*, **437**: 911–915.

Andrade, A., Pablo, J., Espinosa, N., Pe, G. and Gonza, B. (2007). Enzymatic characterization of the enteropathogenic *Escherichia coli* type III secretion ATPase EscN. *Archives of Biochemistry and Biophysics*, **468:** 121–127.

Bansal, G., Ghanem, M., Sears, K.T., Galen, J.E. and Tennant, S.M. (2024). Genetic engineering of Salmonella spp. for novel vaccine strategies and therapeutics. *EcoSal Plus*, **12**(1): eesp00042023

Buttner, D. (2012). Protein export according to schedule: architecture, assembly, and regulation of type III secretion systems from plant- and animal-pathogenic bacteria. *Microbiology and Molecular Biology Reviews,* **76**: 262–310.

Cai, K., Tu, W. and Liu, Y. (2015). Novel fusion antigen displayed-bacterial ghosts vaccine candidate against infection of *Escherichia coli* O157:H7. *Scientific Reports*, **5**:17479.

Chakameh, A.T., Petra, B.W., Ulrike, M., Thomas, S., Matthias, B., John, M.C. and Werner, L. (2004). Bacterial ghosts-biological particles as delivery systems for antigens nucleic acids and drugs. *Frontiers in Microbiology*, **15:** 445.

Chen, P. and Goldberg, M.B. (2023). Recent insights into type 3 secretion system injectisome structure and mechanism of human enteric pathogens. *Current Opinion in Microbiology*, **71**: 102232.

Cornelis, G.R. and Wolfwatz, H. (1997). The Yersinia Yop virulon: a bacterial system for subverting eukaryotic cells. *Molecular Microbiology*, **23**(5): 861-867.

Cortes-Perez, N.G., Lefèvre, F., Corthier,G., Adel-Patient, K., Langella, P. and Bermúdez-Humarán, L.G. (2007) Influence of the route of immunization and the nature of the bacterial vector on immunogenicity of mucosal vaccines based on lactic acid bacteria. *Vaccine,* **25**: 6581-6588.

Dey, S. and Sankaran, S. (2024). Engineered bacterial therapeutics with material solutions. *Trends Biotechnology,* **42**(12): 1663-1676.

Duc, l.H., Hong, H.A., Fairweather, N., Ricca, E. and Cutting, S.M. (2003) Bacterial spores as vaccine vehicles. *Infection and Immunity,* **71**: 2810-2818.

Frahm, M., Felgner, S., Kocijancic, D., Rohde, M., Hensel, M., Rd, C.R., Erhardt, M. and Weiss, S. (2015). Efficiency of conditionally attenuated *Salmonella enterica* serovar Typhimurium in bacterium-mediated tumor therapy. *Mbio*, **6**(2): 55-59.

Ganglberger, E., Barbara, S., Scholl, I., Wiedermann, U., Baumann, S., Hafner, C., et al. (2001). Monovalent fusion proteins of IgE mimotopes are safe for therapy of type I allergy. *FASEB J.***15**: 2524–2526.

Henrich, B., Lubitz, W. and Plapp, R. (1982). Lysis of *Escherichia coli* by induction of cloned phi X174 genes. *Molecular Genetics and Genomics,* **185**: 493–497.

Hu, C.M., Fang, R.H., Wang, K.C., Luk, B.T., Thamphiwatana, S., Dehaini, D., Nguyen, P., Angsantikul, P., Wen, C.H., Kroll, A.V., Carpenter, C., Ramesh, M., Qu, V., Patel, S.H., Zhu, J., Shi, W., Hofman, F.M., Chen, T.C., Gao, W., Zhang, K., Chien, S. and Zhang, L. (2015). Nanoparticle biointerfacing by platelet membrane cloaking. *Nature*, **526**: 118–121.

Ilk, N., Schumi, C.T., Bohle, B., Egelseer, E.M. and Sleytr, U.B. (2011). Expression of an endotoxin-free S-layer/allergen fusion protein in gram-positive *Bacillus subtilis* 1012 for the potential application as vaccines for immunotherapy of atopic allergy. *Microbial Cell Factories,* **10**:6.

Ittig, S.J., Schmutz, C., Kasper, C.A., Amstutz, M., Schmidt, A., Sauteur, L., Vigano, M.A., Low, S.H., Affolter, M. and Cornelis, G.R. (2015). A bacterial type III secretion-based protein delivery tool for broad applications in cell biology. *Journal of Cell Biology,* **211**(4): 913-931.

Jager, J., Marwitz, S., Tiefenau, J., Rasch, J., Shevchuk, O., Kugler, C., Goldmann, T. and Steinert, M. (2014). Human lung tissue explants reveal novel interactions during *Legionella pneumophila* infections. *Infection and Immunity*, **82**: 275–285.

Jahn-Schmid, B., Siemann, U., Zenker, A., Bohle, B., Messner, P., Unger, F.M., sleytr, U.B., Scheiner, O., Kraft, D. and Ebner, C. (1997). Bet v 1, the major birch pollen allergen, conjugated to crystalline bacterial cell surface roteins, expands allergen-specific T cells of the Th1/Th0 phenotype in vitro by induction of IL-12. *International Immunology*, **9**: 1867–1874.

Janeway, C.A. and Medzhitov, R. (2002) Innate immune recognition. *Annual Review Immunology*, **20**:197-216.

Jawale, C.V. and Lee, J.H. (2014). Comparative evaluation of *Salmonella enteritidis* ghost vaccines with a commercial vaccine for protection against internal egg contamination with Salmonella sp. *Vaccine*, **32**: 5925–5930.

Jones, B.D., Pascopella, L. and Falkow, S. (1995). Entry of microbes into the host: Using M cells to break the mucosal barrier. *Current Opinion in Immunology,* **7**(4), 474–478.

Juárez-Rodríguez, M.D., Yang, J., Kader, R., Alamuri, P., Roy Curtiss, I. and Clark-Curtiss, J.E. (2012). Live attenuated salmonella vaccines displaying regulated delayed lysis and delayed antigen synthesis to confer protection against *Mycobacterium tuberculosis. Infection and Immunity,* **80**(2): 815 -831.

Kaufmann, S.H. and Hess, J. (1999). Impact of intracellular location and antigen display by intracellular bacteria: implications for vaccine development. *Immunology Letters*, **65**: 81-84.

Kim, J.H., Yoon, Y.J., Lee, J., Choi, E.J., Yi, N., Park, K.S., Park, J., Lőtvall, J., Kim, Y.K. and Gho, Y.S. (2013). Outer membrane vesicles derived from *Escherichia coli* up-regulate expression of endothelial cell adhesion molecules in vitro and in vivo. *PLoS ONE,* **8**(3) : e59276.

Kimura, K., Iwatsuki, M., Nagai, T., Matsumoto, A., Takahashi, Y., Shiomi, K., Omura, S. and Abe, A. (2011). A small-molecule inhibitor of the bacterial type III secretion system protects against in vivo infection with *Citrobacter rodentium*. *The Journal of Antibiotics (Tokyo)*, **64**, 197–203.

Ko, S.H., Jeon, J.I., Kim, Y.J., Yoon, H.J., Kim, H., Kim, N., Kim, J.S. and Kim, J.M. (2015). *Helicobacter pylori* outer membrane vesicle proteins induce human eosinophil degranulation via a beta2 integrin CD11/CD18- and ICAM-1-dependent mechanism. *Mediators of Inflammation*, **1**: 301716.

Kotton, C.N. and Hohmann, E.L. (2004) Enteric pathogens as vaccine vectors for foreign antigen delivery. *Infection and Immunity,* **72**:5535-5547.

Kwon, S.Y., Thi-Thu Ngo, H., Son, J., Hong, Y. and Min, J.J. (2024). Exploiting bacteria for cancer immunotherapy. *Nature Reviews Clinical Oncology*, **21**(8), 569-589.

Le Gouëllec, A., Chauchet, X., Polack, B., Buffat, L. and Toussaint, B. (2012). Bacterial vectors for active immunotherapy reach clinical and industrial stages. *Human Vaccines and Immunotherapeutics*, **8**(10): 1454–1458.

Liu, Q., Liu, Q., Yi, J., Liang, K., Hu, B., Zhang, X., Curtiss, R. III. and Kong, Q (2016). Outer membrane vesicles from flagellin-deficient *Salmonella enterica* serovar typhimurium induce cross-reactive immunity and provide crossprotection against heterologous Salmonella challenge. *Science Rep*. **6**:34776

Mayr, U.B., Walcher, P. and Azimpour, C. (2005). Bacterial ghosts as antigen delivery vehicles. *Advanced Drug Delivery Reviews,* **57**:1381–1391.

Miao, E.A. and Miller, S.I. (2000). A conserved amino acid sequence directing intracellular type III secretion by *Salmonella typhimurium. Proceedings of the National Academy of Sciences of the USA*, **97**: 7539–7544.

Mori, H. and Ito, K. (2001). The sec protein-translocation pathway. *Trends in Microbiology*, **9**(10): 494-500

Munera, D., Crepin, V.F., Marches, O. and Frankel, G. (2010). N-terminal type III secretion signal of enteropathogenic *Escherichia coli* translocator proteins. *Journal of Bacteriology*, **192**: 3534–3539.

Paccez, J.D., Nguyen, H.D., Luiz, W.B., Ferreira, R.C., Sbrogio-Almeida, M.E., Schuman, W. and Ferreira, L.C. (2007). Evaluation of different promoter sequences and antigen sorting signals on the immunogenicity of *Bacillus subtilis* vaccine vehicles. *Vaccine*, **25**: 4671-4680.

Pais, S.V., Kim, E. and Wagner, S. (2023). Virulence-associated type III secretion systems in gram-negative bacteria. *Journal of Medical Microbiology,* **169**(6):001328.

Panthel, K., Meinel, K.M., Sevil Domènech, V.E., Trülzsch, K. and Rüssmann, H. (2008). Salmonella type III-mediated heterologous antigen delivery: a versatile oral vaccination strategy to induce cellular immunity against infectious agents and tumors. *International Journal of Medical Microbiology*, **298**(1 –2): 99-103.

Rescigno, M., Rotta, G., Valzasina, B. and Ricciardi-Castagnoli, P. (2001). Dendritic cells shuttle microbes across gut epithelial monolayers. *Immunobiology*, **204**: 572-581.

Saggese, A., Baccigalupi, L., Donadio, G., Ricca, E. and Isticato, R. (2023). The bacterial spore as a mucosal vaccine delivery system. *International Journal of Molecular Sciences*, **24**(13), 10880.

Shahid, G.B., Ahan, R.E., Ostaku, J. and Şeker, U.Ö.Ş. (2024). Bacterial living therapeutics with engineered protein secretion circuits to eliminate breast cancer cells. *ACS Synthetic Biology*, **13**(10): 3150–3162.

Sleytr, U.B., Schuster, B., Egelseer, E.M. and Pum, D. (2014). S-layers: principles and applications. *FEMS Microbiology Reviews*, **38**: 823–864.

Toussaint, B., Chauchet, X., Wang, Y., Polack, B. and Le, G.A. (2013). Live-attenuated bacteria as a cancer vaccine vector. *Expert Review of Vaccines*,**12**(10): 1139.

Toyofuku, M., Schild, S., Kaparakis-Liaskos, M. and Eberl, L. (2023). Composition and functions of bacterial membrane vesicles. *Nature Reviews Microbiology*, **21**(7), 415–430.

Trülzsch, K., Roggenkamp, A., Aepfelbacher, M., Wilharm, G., Ruckdeschel, K. and Heesemann, J. (2003). Analysis of chaperone-dependent Yop secretion/ translocation and effector function using a mini-virulence plasmid of Yersinia enterocolitica. *International Journal of Medical Microbiology,* **293**(2): 167-177.

Underhill, D.M. and Goodridge, H.S. (2012). Information processing during phagocytosis. *National Reviews of Immunology,* **12**: 492–502.

Uyen, N.Q., Hong, H.A. and Cutting, S.M. (2007). Enhanced immunisation and expression strategies using bacterial spores as heat-stable vaccine delivery vehicles. *Vaccine*, **25**: 356-365.

Woltjen, K., Michael, I.P., Mohseni, P., Desai, R., Mileikovsky, M., Hamalainen, R., Cowling, R., Wang, W., Liu, P., Gertsenstein, M., Kaji, K., Sung, H.K. and Nagy, A. (2009). piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. *Nature*, **458**(7239): 766 -770.

Worrall, L.J., Majewski, D.D. and Strynadka, N.C.J. (2023). Structural insights into type III secretion systems of the bacterial flagellum and injectisome. *Annual Review of Microbiology.*

Yu, X., Wang, L., Yang, X., Zhang, S., Li, G., Zhang, L., Li, J., Wang, X., Zhou, H., Jiang, Y., Cui, W., Li, Y., Tang, L. and Qiao, X. (2022). *Lactobacillus casei* ghosts as a vehicle for the delivery of DNA vaccines mediate immune responses. *Frontiers in Immunology,* **13 :** 849409.

Yuan, C., Ji, X., Zhang, Y., Liu, X., Ding, L., Li, J., Ren, S., Liu, F., Chen, Z., Zhang, L., Zhu, W., Yu, J. and Wu, J. (2024). Important role of *Bacillus subtilis* as a probiotic and vaccine carrier in animal health maintenance. *World Journal of Microbiology and Biotechnology*, **40:**268.

Zhang, H., Li, W., Li, Y., Wang, Y., Jin, Y., Tong, D., Li, Z. and Zhou, J. (2024). Bacterial ghosts engineered with lipidated antigens as an adjuvant-free vaccine for *Chlamydia abortus*. *International Journal of Pharmaceutics*, **666**: 124801.

Zurawski, D.V. and Stein, M.A. (2003). SseA acts as the chaperone for the SseB component of the Salmonella pathogenicity island 2 translocon. *Molecular Microbiology*, **47**: 1341–1351.