Biological Times

Volume No. 5 | Issue No. 1 | Pages 21-22

The use of Escherichia coli in the Administration of Therapeutic Proteins

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ABSTRACT

Escherichia coli Nissle is a probiotic strain, which is engineered to administer drugs and proteins locally, reducing drug degradation and dilution, and for target-specific therapy. There are 4 Methods discussed here: "Outer Membrane Vesicles" OVMs, Curli fiber-mediated for inflammatory gut disease, and Targeted expression of certain recombinant gene upon detection of certain chemical signals, and lastly, Bacterial Ghost for administration of Antibodies and Antigen safely, and for previously deemed ineffective drugs due to its structural similarity to ineffective anti cancerous drug. This might seem like a cheat code in diagnosis and treatment, but it can be pathogenic for immunocompromised ones, can disrupt the genome of normal host cells, can cause toxicity, and can become resistant to antimicrobials due to horizontal gene transfer.

Keywords: EcN, Therapeutics, OVMs, Drug administration, curli fiber

To cite this article: Babur A, U Babur, R Zulfiqar, SUD Afghan & A Jahangir. The use of Escherichia coli in the Administration of Therapeutic Proteins. Biological Times. 2026. January 5(1): 21-22.

Introduction

E.coli is used as a model bacterium in bacteriology because it is easy to grow, genetically manipulable, is cheap, and replicates very fast. It is used as a model to understand the basic biological pathways and processes of bacteria, such as cell division and growth, gene regulation, stress response, and the central dogma of molecular biology, aside from that, *E. coli* is also used to discover foundational concepts of genetics like operons, gene mapping, mutations, and selections, and it exists as a probiotic as well as a pathogen for us. It can be redesigned to produce Amino acids, Vitamins, Organic acids, Bioplastics, etc. [3]. And the reason it is loved by bacteriologists is that it replicates very fast (around 20-30 mins per cell division), is easy to culture, and has predictable behavior in experiments [11].

A probiotic strain, "*E.coli* Nissle 1917(EcN)" is engineered to deliver therapeutic proteins [13]. Because of its probiotic nature and ability to colonize the gut, which is necessary for the administration of therapeutic protein via the Gut barrier. On the other hands the *E.coli* BL21 and the K-12 strains are used for the manufacturing of such proteins [12]. Different methods have been discovered by which *E.coli* is capable of transporting such proteins as OVMs.

OMVs mediated delivery

Outer membrane vesicles (OVMs) are secreted by Gram-negative bacteria. A strain of *E.coli* was engineered to carry therapeutic proteins, such as Antigens and antibodies, inside or outside the OVMs to transport them safely to the blood so that the antigens and antibodies can control the infection by stimulating inflammation and by neutralizing pathogens. This method is deemed useful as an antigen can be transported while expressed onto the surface of the OMV and acts as an attenuated pathogen, which stimulates an immune response to get immunity against that certain pathogen, and can be useful for vaccine development and administration[1]. Similarly, in the case of certain antibodies that have poor oral administration, these antibodies and other proteins like insulin can be transported encased by OVMs to avoid being degraded by GIT enzymes [2].

Curli Fiber mediated

Curli Fibers are naturally present on some *E.coli* as extracellular protein fibers used for adhesion and attachment to surfaces and for biofilm formation. It consists of two subunits, CsgA and CsgB. CsgA is the main subunit, while CsgB is like an anchor. They make a net outside the cell. CsgA is fused with our protein of interest, and on adhesion to the GIT, the protein of interest which is often a toxin neutralizer or promotes wound healing, and delivers antigens too, lastly kills worn-out enzymes in inflammation, thus helping the body to recover from infection. Like Variable Heavy-chain domain of Heavy-chain only antibodies" VHH, an antibody extracted from camels is used to block the virulence of Enteric pathogens like EPEC, Shigella, *Clostridium difficile* etc [5]. Another important example is the Curli-trefoil complex, in which the trefoil protein, which is naturally produced by the gut to treat minor damages, but in case of IBD and other severe damages to the bowel, Trefoil is artificially given via the curli fiber system [4].

Targeted expression and excretion of proteins via sensing

The EcN strain is genetically modified in such a way that upon reaching the gut, it senses a chemical signal and starts forming therapeutic proteins that are administered locally, so it acts as a biochemical diagnostic-therapeutic bio-machines. It can effectively control gut infections and necrosis. In the case of ulcerative colitis, "PS" is exposed on apoptotic colon cells. The engineered EcN with "Annexin A5" (ANXA5) surface displaying protein, this protein binds with PS and helps the engineered EcN to colonize with apoptotic cells, and after colonization, it releases "anti TNFα nanobody" which decreases the inflammation and ultimately heals the mucosa [6]. Another example of this method is that high concentrations of Nitric Oxide are detected by NorR, a transcription-based factor of our recombinant agent, and it uses its Type-1 hemolysin secretion system to secrete anti-TNFα nanobodies to reduce inflammation [7].

Bacterial Ghost cells

The concept is very simple: the empty bacterial capsule filled with our proteins, which can be antigens or antibodies too are delivered orally, and the bacterial cell wall endures the harshness of the gut environment, keeping the proteins safe. For instance, the EcN strain, which is a probiotic strain, was treated with Triton X-100 and NaCl; this changed the cell membrane structure and made it permeable; however, this was further enhanced by using heat-shock treatment to remove all cytoplasmic material, including the genome, and killing the organism. The viability test, cell leakage test, live-dead cell test, and scanning electron microscopy analysis were used to evaluate this method's effectiveness. And then these Bacterial Ghosts were filled with "ESAT-6", which is a *Mycobacterium tuberculosis* antigen [9]. In another study, EcN was used as a bacterial ghost to deliver an anti-cancer drug, " benzimidazole-based Pt(II)-N-heterocyclic carbene (NHC) complexes", which is a Platinum(II)-NHC drug. It is similar to Cisplatin and Carboplatin. The EcN shells acted as Trojan horses and killed cisplatin resistant tumor and have better selectivity for cancerous and normal cells.

Limitations:

Although EcN based protein administration is very promising, it is not 100% risk free since it can:

Become a resistant strain by horizontal gene transfer. The LPS endotoxins of bacterial ghosts cause toxicity, have the potential to be persistent and escape, are not fully reliable for biocontainment, and are subject to quality control mismanagement and mutations due to normal microbiota, overexpression, and colonization, and could be Bio-pollution [10] .

Future Prospects or Applications

Since EcN is a probiotic bacterium, it can be engineered to produce useful peptides, vitamins, and amino acids that are essential for our body and required for the specific metabolic pathway. It can also be improved to do precision cancer therapy with anticancer drugs targeting novel neoantigens and destroying their host cells. It can be developed to release specific therapeutic proteins and drugs upon sensing specific chemical signals, and uses of medicine for common infections can be minimized to reduce cost and manage AMR.

Conclusion

EcN is a gateway for multiple biotechnical developments, just like the subject of this article. OVMs are used to deliver proteins safely and protect them against proteases in the gut. While Curli Fibers help to control inflammation in the gut due to foodborne pathogens and apoptotic cells, and block both the pathogens and suicidal cells. Targeted expression upon sensing is as simple as EcN recognizes chemical signals, it starts the production and extraction of our required recombinant protein. In the case of Bacterial Ghost, empty bacterial shells are used to transport proteins. It has enormous potential for the future of Molecular Biology and recombinant gene technology. Diagnosis and treatment are now much more convenient, efficient, and precise, and are subject to further improvement.

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