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Review Article

LIVE BIOTHERAPEUTIC PRODUCTS AS A MODERN BIOTECHNOLOGICAL STRATEGY AGAINST ANTIMICROBIAL RESISTANCE

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ABSTRACT. This article addresses the growing global threat of antimicrobial resistance (AMR) and examines Live Biotherapeutic Products (LBPs) as a modern and sustainable biotechnological strategy to combat it. AMR arises through multiple molecular mechanisms, including efflux pumps, enzymatic drug degradation, biofilm formation, target modification, decreased membrane permeability, and horizontal gene transfer, all of which contribute to the failure of conventional antibiotics and the spread of multidrug-resistant pathogens. LBPs are defined as regulated biological medicinal products that contain live microorganisms and are designed to prevent, treat, or manage diseases. Unlike conventional antibiotics, LBPs act through biological and ecological mechanisms rather than chemical killing, thereby reducing selective pressure for resistance. They inhibit pathogens by competing for nutrients and adhesion sites, producing antimicrobial agents such as bacteriocins, disrupting biofilms, and modulating host immune responses. The article highlights the critical role of biotechnology in LBP development, including genetic engineering, synthetic biology, metagenomic analysis, controlled fermentation, and encapsulation technologies. These tools enable the design of safe, targeted, and effective therapeutic microbes with improved stability and clinical performance. Furthermore, regulatory and safety considerations are addressed, encompassing preclinical studies, clinical trials, and quality control requirements enforced by the FDA and EMA. Major challenges such as biosafety, dosage standardization, and storage stability are also discussed. In conclusion, LBPs represent a promising next-generation approach to AMR by integrating microbiology and precision medicine, expected to play a central role in future global health strategies.

Keywords: *Antimicrobial Resistance (AMR), Biofilms, Horizontal gene transfer, Live Biotherapeutic Products (LBPs), Microbiome, Synthetic Biology.*

INTRODUCTION

Antimicrobial Resistance (AMR) is the ability of microorganisms, such as bacteria, viruses, fungi, and parasites, to survive and grow despite exposure to antimicrobial drugs that were previously effective against them. As a result, standard treatments become ineffective, infections persist, and the risk of spread increases. Antimicrobial resistance has become one of the most serious threats to global health. The increasing failure of conventional antibiotic treatments has created an urgent need for innovative and sustainable alternatives. Live Biotherapeutic Products (LBPs) have emerged as a new generation of biological strategies that use live microorganisms as therapeutic agents to prevent, treat, or manage diseases [1].

The World Health Organization (WHO) has characterized antibiotic resistance as a global crisis, warning of the potential for a post-antibiotic era where widespread infections and minor injuries could once again become fatal due to the lack of effective

treatments. The molecular mechanisms by which bacteria acquire and spread drug resistance are varied and highly effective [2].

These mechanisms include spontaneous genetic mutations that alter the target sites of antibiotics, activation of efflux pumps that expel antibiotics from bacterial cells, and the production of enzymes that break down or inactivate the drugs. Bacteria also adopt physical strategies such as biofilm formation, which coats bacterial communities with protective matrices, making it difficult for antibiotics to penetrate and act effectively [3]. However, one of the strongest drivers of resistance is horizontal gene transfer, where bacteria exchange genetic material, including resistance genes, between species and strains. Horizontal Gene Transfer (HGT) facilitates the rapid spread of resistance traits, leading to the emergence of multidrug-resistant and even globally resistant bacterial strains. In light of these challenges, traditional approaches to antimicrobial therapy are becoming increasingly inadequate, prompting researchers to seek innovative solutions [4].

OVERVIEW OF ANTIMICROBIAL RESISTANCE

Main Causes of Antimicrobial Resistance

One of the main causes of antibiotic resistance is the misuse and overuse of antimicrobials in human medicine. Antibiotics are often prescribed unnecessarily, used for viral infections, or taken incorrectly, such as prematurely discontinuing treatment; this allows resistant microorganisms to survive and multiply. Another important factor is the widespread use of antimicrobials in agriculture and animal husbandry, where they are often applied not only for treatment purposes but also for disease prevention and growth promotion. This practice accelerates the emergence of resistant strains that can be transmitted to humans through the food chain or the environment. Inadequate infection prevention and control measures, such as poor hygiene and sanitation, particularly in healthcare facilities, facilitate the spread of resistant pathogens. Additionally, environmental contamination with antimicrobial residues from pharmaceutical production, hospitals, and agricultural runoff creates a selective pressure that promotes resistance in environmental microorganisms. Furthermore, global travel and trade play a significant role in the rapid international spread of resistant microorganisms. These factors, combined with genetic mechanisms such as mutations and horizontal gene transfer, ensure the persistence and spread of resistance genes among microbial populations [1,5].

Molecular Mechanisms of Antimicrobial Resistance

The molecular mechanisms of drug resistance in bacteria are diverse and complex. They can be divided into several categories, each involving different strategies that bacteria use to survive in the presence of antibiotics. Drug resistance in bacteria is a significant global health problem with mechanisms that may include 1) Emissivity pumps, these proteins actively expel antibiotics from bacterial cells, reducing drug concentration to non-lethal levels [6]. 2) Enzymatic degradation or modification, bacteria produce enzymes such as beta-lactamases that can render antibiotics ineffective [7]. Mutations alter the bacterial components targeted by the antibiotic, making it less effective [8]. 3) Biofilm formation, bacteria in biofilms are more resistant to antibiotics due to reduced penetration and slowed growth rates [9]. Genes responsible for

resistance can be transferred between bacteria via plasmids, transposons, or bacteriophages [4].

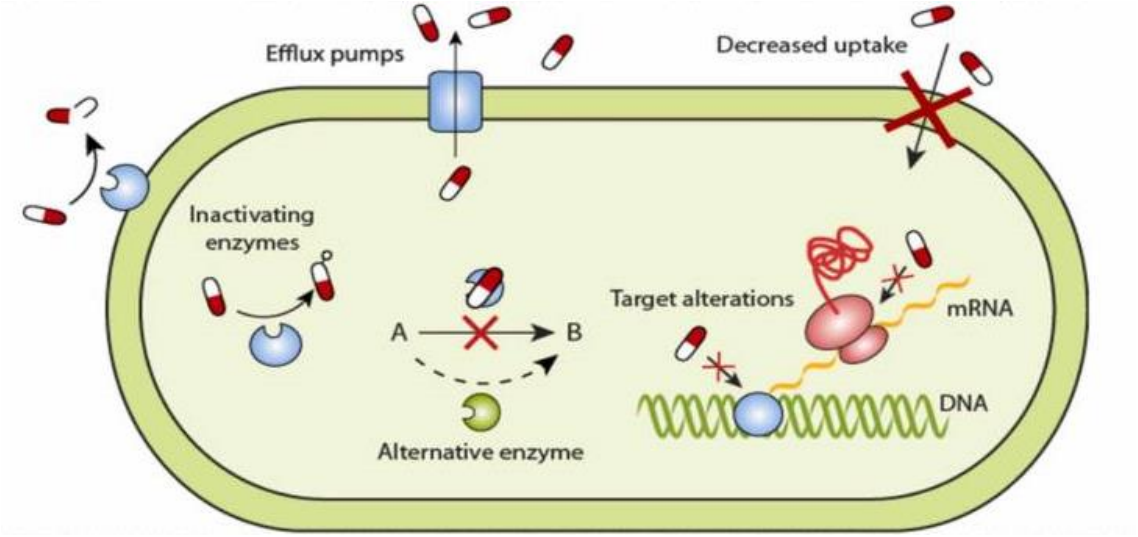


Fig. 1. *Molecular Mechanism of Antibiotic Resistance in Bacteria*

MECHANISM OF ANTIBIOTIC RESISTANCE IN BACTERIA

Eflux Pumps

One of the most common and versatile mechanisms of drug resistance. Efflux pumps contribute significantly to bacterial drug resistance due to their ability to actively remove antibiotics from the cell, thereby reducing intracellular antibiotic concentration. Efflux pumps prevent the drug from reaching its target by reducing the drug concentration within the bacterium to non-lethal levels. Efflux pumps are transport proteins (membrane-bound proteins) found in bacterial cells and typically use energy in the form of ATP hydrolysis or proton force to pump harmful substances out [10, 11].

Enzymatic Degradation of Drugs

Enzymatic degradation is a complex process carried out by enzymes and microorganisms such as bacteria and fungi [12]. Furthermore, enzymatic degradation depends on a combination of internal/external and biotic/abiotic factors. Temperature and pH (which affect microbial activity), microorganism species, and polymer surface are important in this process. Microorganisms move outside the cell, breaking down polymer molecules and preparing them for further degradation by intracellular enzymes [13]. Enzymatic degradation occurs through the following stages; (i) Attack of microorganisms on the polymer surface; (ii) Depolymerization; (iii) Absorption and metabolism (primary degradation); (iv) Mineralization (secondary degradation). The process begins with the adhesion of microorganisms to the hydrophilic surface of the polymer and the secretion of extracellular enzymes responsible for the depolymerization

of macromolecular chains. Extracellular enzymes break the chains, making the polymer surface even more hydrophilic and therefore more susceptible to invasion by other microorganisms [14, 15]. During primary degradation, low molecular weight products are assimilated by microorganisms and converted into biomass. In the secondary degradation process, this biomass is further converted into byproducts [16]. The absorption and mineralization of polymer chains by microorganisms can occur in the presence or absence of oxygen. When the process takes place in a well-oxygenated environment (O_2 concentration at least 6%), aerobic biological degradation is achieved with the production of carbon dioxide (CO_2) and water (H_2O) as the main digestive products. Anaerobic decomposition can occur under both mesophilic (37 °C) and thermophilic (55 °C) conditions [17].

Biofilm Formation

Reduced antibiotic penetration occurs because biofilms form a protective barrier that prevents antibiotics from reaching all bacterial cells within the biofilm. This physical barrier makes it difficult for drugs to completely eliminate the bacteria, slowing the growth rate of bacteria within the biofilm, often leading them into a slow-growing or dormant state. Many antibiotics target actively dividing cells, so this slow growth can make bacteria less susceptible to treatment. Biofilm bacteria communicate using Quorum Sensing (QS), which can regulate the expression of resistance genes [18]. This can lead to coordinated responses to stress, including antibiotic pressure. The proximity of bacteria in biofilms facilitates the transfer of genetic material, including antibiotic resistance genes, through (HGT). This means that resistant bacteria within a biofilm can share resistance traits with neighboring cells. Biofilms often harbor a small subpopulation of bacteria (persistent) that can survive antibiotic treatment due to their dormant state. After antibiotic treatment ends, these cells can repopulate the biofilm, leading to chronic or recurrent infections [19].

Target Modification

Target modification serves as an essential method through which bacteria acquire resistance to antibiotics. This approach entails changes to the elements within bacteria that antibiotics aim to affect, reducing the efficacy of the medications or rendering them entirely powerless. Target modification involves changes in the structure or chemistry of the bacterial molecules that antibiotics attach to, which can include ribosomes, enzymes, or components of the cell wall. When an antibiotics intended target is altered, the antibiotic loses its capacity to effectively bind, rendering it unable to stop bacterial proliferation or eliminate the bacteria. Target modification significantly contributes to resistance against various antibiotic categories, such as beta-lactams, fluoroquinolones, macrolides, and glycopeptides. It functions modified target sites reduce the antibiotics binding strength, making it challenging for the drug to produce its effects. Certain bacteria adapt their target molecules so they can continue to perform their normal roles while becoming more resistant to the antibiotic's effects. Alterations in target sites can also result in cross-resistance, leading bacteria to resist multiple antibiotics that target similar sites [20].

Reduced Drug Permeability

Reduced permeability is a mechanism by which bacteria develop resistance to antibiotics, decreasing the ability of drugs to penetrate the bacterial cell. This mechanism primarily involves changes in the bacterial cell membrane or cell wall that limit the entry of antibiotics into the cell, thus reducing their intracellular concentrations [21].

Reduced permeability is particularly important in resistance to hydrophilic antibiotics that require porin channels to enter the bacterial cell, such as beta-lactams, tetracyclines, and some fluoroquinolones. The main ways it contributes to resistance are when fewer or modified porins are present, the antibiotic cannot effectively enter the bacterial cell, leading to intracellular drug concentrations below therapeutic levels. Reduced permeability often works in conjunction with other resistance mechanisms, such as efflux pumps or enzymatic degradation, increasing the ability of bacteria to survive in the presence of antibiotics. This mechanism helps bacteria survive in challenging environments where antibiotics are present, contributing to the persistence and spread of their resistance [22].

Horizontal Gene Transfer

In bacteria, the spread of antibiotic resistance, particularly in healthcare settings, is largely due to (HGT). Multidrug-resistant pathogens can arise as a result of horizontal gene transfers ability to rapidly acquire new DNA sequences, such as Antimicrobial Resistance Genes (ARGs). This problem is particularly concerning when it comes to hospital-acquired infections, as resistant *Acinetobacter baumannii* can cause mortality rates to rise to 19–54%. While horizontal gene transfer promotes the spread of resistance, it is interesting that some bacterial defense mechanisms can slow down this process [23]. Bacteria acquire free DNA from their environment (usually from lysed cells) and integrate it into their own genomes. This process can allow bacteria to acquire resistance genes from dead or dying bacteria [4].

LIVE BIOTHERAPEUTIC PRODUCTS

Live Biotherapeutic Products (LBPs) are medicinal products containing live microorganisms, such as bacteria, designed for use in the prevention, treatment, or cure of a disease; they are not vaccines. Unlike traditional probiotics, LBPs are regulated as biological drugs and must undergo rigorous clinical evaluation. Live biotherapeutic products utilize the interaction between the administered live microbes and the host body (typically by modifying the natural microbiome) to achieve the desired therapeutic effect, such as treating infections, inflammatory bowel diseases, or other conditions. A live biotherapeutic product consists of genetically modified microorganisms through the intentional addition, deletion, or alteration of genetic material. In contrast to live biotherapeutic products, prebiotics, probiotics, and synbiotics are widely used as functional foods and nutritional supplements without requiring regulatory approval and lack robust clinical trial data on their effectiveness in preventing and treating any specific disease [24].

The Relationship Between Live Biotherapeutic Products and Antimicrobial Resistance

Live biotherapeutic agents combat antimicrobial resistance through biological mechanisms rather than chemical degradation. They suppress pathogenic bacteria by competing for nutrients, producing antimicrobial agents, stimulating host immunity, and disrupting bacterial biofilms. These mechanisms significantly reduce the risk of resistance development. *Lactobacillus bacteriocins* (live biotherapeutic agents) offer a novel and promising approach to combating Antimicrobial resistance by utilizing complex biological mechanisms instead of relying solely on the chemical degradation properties of conventional antibiotics. These ribosomally synthesized peptides, produced by *Lactobacillus* species, suppress the growth and virulence of pathogenic bacteria through various key actions that significantly reduce the selection pressure for resistance development [25].

Antimicrobial Resistance Mechanisms of Live Biotherapeutic Products

Competition for nutrients and adhesion sites, *Lactobacillus* strains that produce live biotherapeutic products effectively compete with pathogens for essential nutrients (such as carbohydrates and amino acids) and adhesion sites on mucosal surfaces (e.g., intestinal or vaginal tract). This competitive exclusion prevents the colonization and proliferation of harmful and potentially resistant bacteria. Production of antimicrobial agents (bacterocins), the primary weapon is the production of bacteriocins, which are potent and narrow-spectrum peptides. These peptides typically target the cell membranes of susceptible bacteria, particularly gram-positive pathogens. Many live biotherapeutic agents (such as nisin) bind to the bacterial cytoplasmic membrane, forming pores or channels. This disrupts the membrane potential, leading to leakage of essential intracellular components (adenosine triphosphate, ions) and ultimately death (lysis) of the bacterial cell. Bacteria are more difficult to overcome this physical mechanism than through chemical inhibition. Breakdown of bacterial biofilms, biofilms which are complex and protective microbial communities, contribute significantly to antimicrobial resistance as they protect bacteria from both antibiotics and the host immune system. Live biotherapeutic agents can inhibit biofilm formation by preventing the initial attachment of pathogenic bacteria to surfaces and disrupting mature biofilms, disrupting the Extracellular Polymeric Substance matrix(EPS), or killing embedded cells, making pathogens more vulnerable to clearance. Specific live biotherapeutic products and *Lactobacillus* strains interact with the host’s immune cells. They can influence the immune response by stimulating the production of anti-inflammatory cytokines and enhance the body's ability to eliminate infections without the need for chemical treatments, thereby enhancing its ability to eliminate pathogens. The diverse and often membrane-affecting properties of (LBPs) and their low likelihood of developing resistance compared to existing antibiotics make them a fundamental biodiversity-focused approach in the fight against antimicrobial resistance worldwide [26, 27, 28].

THE ROLE OF BIOTECHNOLOGY IN THE DEVELOPMENT OF LIVE BIOTHERAPEUTIC PRODUCTS

Biotechnology plays a significant role in the development of live biotherapeutic products through genetic engineering, metagenomic analysis, controlled fermentation,

encapsulation technologies, and bioprocess optimization. Advanced techniques allow scientists to design bacteria that produce therapeutic molecules directly inside the human body [29].

Genetic Engineering (Synthetic Biology)

Synthetic biology is a new interdisciplinary science involving the synthesis of biological components, systems, and organisms using genetic engineering technology. Manipulating DNA or transferring the DNA of one organism to another leads to synthetic biology. Manipulating the genomes of organisms is revolutionizing the genetics of organisms. Cloning genes is a significant genetic engineering technology and is essential for studying the biological characteristics of genes, DNA fragments, and the organization of genomes. Other techniques that are part of genetic engineering technology include DNA isolation, restriction digestion of DNA, ligation, sequence analysis, and expression [30]. The most advanced application going beyond simple microbial cultures 1)Engineering therapeutic microbes, biotechnology allows for the precise modification of bacterial genomes, enhancing their therapeutic properties, or even creating new functions 2)Targeted production, scientists can engineer bacteria to produce specific therapeutic molecules in situ (directly inside the human body, usually the gut). For example, microbes can be designed to produce cytokines that regulate immune responses and enzymes that break down toxins or problematic metabolites 3)Antimicrobial peptides that selectively target pathogens 4)Safety and regulation, genetic "kill switches" or systems for controllable gene expression within bacteria can be designed, thus allowing therapeutic efficacy to be controlled, triggered, or stopped [31].

Metagenomic Analysis and Bioinformatics

Metagenomics is a rapidly developing field of study that is revolutionizing our understanding of microbial communities and their immense biodiversity. Unlike traditional microbiology, which relies on culturing isolated strains, metagenomics involves the direct genetic analysis of all microbes present in an environmental sample (such as soil, water, or the human gut). This aggregated genetic material is often referred to as the metagenome. This field is crucial for the initial discovery and characterization phase [32].

Metagenomics results in the identification of different strains, involving the direct analysis of genetic material from environmental sources such as the skin or gut microbiome. This method allows researchers to find and characterize beneficial bacterial species and strains with advantageous therapeutic properties. Understanding mechanisms of action by analyzing the entire genetic potential of a microbial community, scientists can identify specific genes and pathways (e.g., colonization resistance, short-chain fatty acid production) responsible for the effects of living biotherapeutic products. Bioinformatics tools are used to screen large amounts of genomic data in order to select the most robust, safe, and effective candidate strains for live biotherapeutic product development [33].

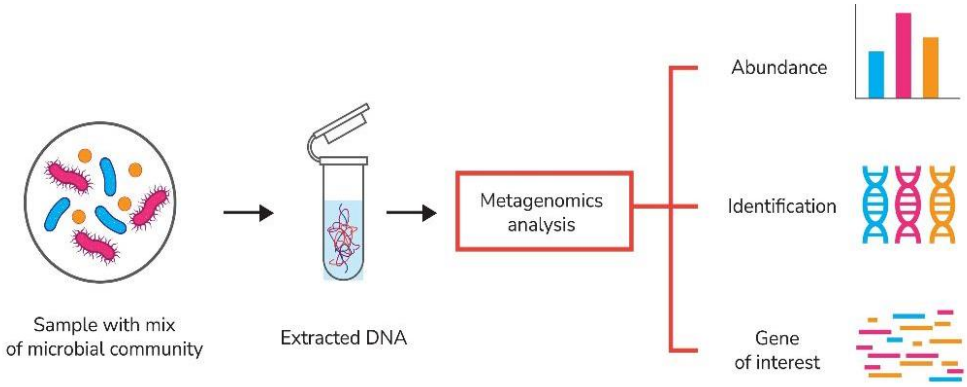


Fig. 2. *Metagenome Analysis*

Controlled Fermentation (Upstream Processing)

Producing live biotherapeutic products on a large scale requires advanced bioprocessing methods. A key element of this procedure is bioreactor optimization, which involves the use of specialized bioreactors to cultivate selected microbial strains under tightly regulated factors such as temperature, pH, nutrient source, and oxygen concentration. This ensures high yield, viability, and product purity. Furthermore, biotechnology plays a significant role in formulating chemically defined, customized fermentation media suitable for specific strains, enhancing the growth and therapeutic efficacy of the live biotherapeutic product strain while reducing contamination risks. Maintaining aseptic conditions and adhering to Good Manufacturing Practices (GMP) standards throughout the fermentation process is vital to ensure the final product remains free from contamination and maintains consistent quality from one batch to the next [34].

Encapsulation Technologies and Formulation (Downstream Processing)

The efficient transport of live biotherapeutic products to their intended site of action, typically the large intestine, is a crucial factor influencing their efficacy. Various formulation techniques are employed to facilitate this process. Since live microorganisms are easily affected by harmful factors such as stomach acid, bile salts, and physical challenges encountered during transport, protecting the products from the aggressive conditions of the gastrointestinal system is vital. Advanced encapsulation methods, such as hydrogels or polymeric materials, are used to protect live biotherapeutic products and maximize their survival rates once they reach their target location. Furthermore, formulation researchers focus on controlled release mechanisms, developing specialized delivery systems that allow for delayed or targeted release of bacteria. These systems are typically activated when live biotherapeutic products encounter a specific pH level, such as the distal small intestine or colon. Ultimately, enhancing stability is vital to ensure the product remains viable and effective while extending its shelf life. Methods such as lyophilization (freeze-drying) are frequently

used to stabilize bacteria, ensuring that the live biotherapeutic product maintains its effectiveness throughout its storage period [35].

CRISPR-Cas Technology in Microbial Engineering

The swift progress of LBPs has been greatly enhanced through the use of CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats) technology, which provides a highly adaptable and accurate method for genome alteration in therapeutic bacteria. In contrast to conventional genetic modification approaches, CRISPR-Cas systems act as revolutionary mechanisms for tackling AMR by functioning as targeted antimicrobials capable of being introduced into engineered probiotics that aim to identify and eradicate particular resistance genes or virulence factors present in pathogenic groups without negatively impacting the helpful commensal microbiota. Additionally, this technology plays a crucial role in improving both the effectiveness and safety of LBPs, as it facilitates the exact removal of inherent antibiotic resistance genes from the therapeutic strains and permits the reliable incorporation of therapeutic elements and genetic 'kill switches' to guarantee effective biocontainment and adherence to regulations. As a result, the accuracy provided by CRISPR-based modifications offers an essential basis for creating the forthcoming generation of 'smart' probiotics that are not only highly effective but also aligned with biosafety standards in combating multi-drug resistant pathogens [36, 37].

Bioprocess Optimization and Analytics

Bioprocess optimization and analytics involves the systematic improvement of biological production systems through data-driven approaches, mathematical modeling, and continuous monitoring to increase yield, efficiency, quality, and cost-effectiveness in biotechnological production. This improvement relies heavily on vital methods such as Process analytical technology, where biotechnology devices like state-of-the-art sensors and spectroscopy techniques are incorporated into the production process, thus ensuring consistency in the product. Additionally, scaling up is crucial and requires expertise in bioprocess engineering to move the production of a viable strain of the LBP from a small laboratory scale to a larger production level without losing its therapeutic qualities. Finally, effective Quality Control (QC) is achieved through methods such as Flow Cytometry and quantitative polymerase Chain Reaction, which allow for the rapid and accurate counting of live cells and meticulously verify the identity and purity of the live biotherapeutic product [38].

THE ROLE OF THE HUMAN MICROBIOME IN PATHOGEN RESISTANCE AND ITS APPLICATION IN LIVE BIOTHERAPEUTIC PRODUCTS

The human microbiome can confer resistance to pathogens developing infections through both direct and indirect pathways. Without host intervention, the microbiome can directly kill pathogens by producing metabolites and toxins and depriving pathogens of essential nutrients [39]. For example, the vaginal microbiome is predominantly composed of lactic acid-producing *Lactobacillus* species[40].

The acidic vaginal environment inhibits the growth of harmful bacteria such as *Escherichia coli*, *Neisseria gonorrhea*, and *Chlamydia trachomatis*. Lactic acid can also aid in preventing viral infections by preventing the binding of the Human Immunodeficiency Virus (HIV) surface payload to the host cell through inactivation. Indirectly, the host microbiome can activate innate and adaptive immune responses and stimulate the production of anti-pathogen metabolites to confer resistance to pathogenic infection. Common skin colonizers such as *Staphylococcus epidermidis* can induce CD8 T cells, which provide antimicrobial protection and aid in tissue repair [40].

Similar to the host microbiome, live biotherapeutic products can be a single microbial strain or a community of microbial strains designed to interact with the host ecosystem through innate microbial characteristics or genetic modification [41]. These designed live biotherapeutic products can function by inhibiting the growth of pathogenic organisms or toxins, stimulating the production of beneficial molecules to prevent cancer, and altering metabolism and the mucosal immune system to reduce inflammation [42].

A study on human colon explants pretreated with a single-strain live biotherapeutic product (SS-live biotherapeutic product) of *Bacillus velezensis* strain ADS024 and subsequently exposed to *Clostridium difficile* toxin demonstrated that protease secretion by ADS024 led to lower toxin B levels and toxin-mediated cell apoptosis [43]. Therefore, the SS-live biotherapeutic product has the potential to protect against colonic mucosal damage after standard antimicrobial treatment and to prevent recurrent *Clostridium difficile* infection.

REGULATORY AND SECURITY CONSIDERATIONS

Live biotherapeutic products must undergo preclinical testing and clinical trials (Phase I, II, and III) prior to regulatory approval. Strict quality control, safety assessment, and post-marketing surveillance are necessary to ensure their safe use. Major regulatory bodies such as the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) require a comprehensive assessment based on three key elements: Quality, Safety, and Efficacy [44].

Preclinical Testing and Research New Drug Application

Sponsors are required to submit an Investigative New Drug (IND) Application to the relevant regulatory authority, such as the FDA's Center for Biological Evaluation and Research (CBER), before commencing human trials for a LBP. The IND application must include several key elements, strain characterization, requiring a detailed identification and examination of the microbial strain(s), often involving whole-genome sequencing, with regulators specifically looking for the absence of virulence factors and transferable antibiotic resistance genes to prevent the spread of resistance; safety assessment, involving preclinical (animal) studies where standard ADME (Absorption, Distribution, Metabolism, Excretion) assessments are often inappropriate because LBPs are generally not intended for systemic circulation; therefore, research emphasizes factors such as colonization, shedding, and elimination from the host, such as in the gut. Finally, Chemistry, Manufacturing, and Controls (CMC), representing a critical and

complex aspect requiring comprehensive documentation to demonstrate the quality and consistency of the product. This includes establishing and evaluating a Master Cell Bank (MCB) and a Working Cell Bank (WCB) according to Good Manufacturing Practices (GMP) standards to provide a stable and uncontaminated source for production, and process validation to record how production methods (such as fermentation and lyophilization) consistently maintain strain viability and prevent contamination [24].

Clinical Trials (Phases I, II, and III)

Clinical trials follow the standard drug development structure to demonstrate that live biotherapeutics have a favorable risk-benefit balance. Phase I (Safety), the primary goal is to validate safety and determine the maximum dose or appropriate biological dose that can be safely administered in a small group of healthy volunteers or patients, and to assess any adverse reactions such as systemic infections, toxin production, or unwanted microbial activity. Phase II (Efficacy and Safety), the goal is to gather preliminary data on the efficacy of the treatment (does it work?) and to continue assessing safety in a larger patient group while determining the optimal dosage strategy and demonstrating a measurable biological effect (e.g., changes in the structure or activity of the patient's microbiome). Phase III (Validation of Efficacy and Safety), the goal is to evaluate the efficacy and safety of the live biotherapeutic product in a large and diverse patient group and compare it to a placebo or established treatment. This stage emphasizes the submission of conclusive evidence of clinical benefit required for regulatory approval (Marketing Authorization Certificate/Biological Product License Application) [44, 45, 46].

Post-Marketing Surveillance and Quality Control

Post-marketing surveillance is the continuous monitoring of a product's safety, quality, and efficacy after it has been approved and launched on the market. It allows for the identification of unexpected risks, long-term effects, or quality deficiencies in real-world use. This can help detect rare side effects that may not have appeared in clinical trials (FDA, WHO, EMA).

CHALLENGES IN DEVELOPING LIVE BIOTHERAPEUTIC PRODUCTS

Limitations of Classical Culture-Based Methods for Bacterial Viability Assessment

Traditional techniques for assessing bacterial viability, particularly through colony counting on solid media, are widely used but often underestimate the true number of viable microorganisms. This drawback poses a significant obstacle, especially in the development of Live Biotherapeutic Products where accurate measurement of viability is crucial for maintaining product quality, safety, and efficacy. Standard culture methods frequently underestimate the number of viable cells because they fail to detect non-lethal damaged cells that cannot multiply, and bacteria that are difficult or impossible to culture, such as those in the Viable But Not Culturable (VBNC) phase. To overcome these challenges, new, culture-independent approaches have been developed that highlight different indicators of cellular health, focusing primarily on cellular integrity,

which refers to a complete cellular structure, and cellular activity, which encompasses metabolic processes or physiological responses. Research comparing older and newer methods of assessing viability shows notable differences; for example, Live/Dead staining frequently detects viable cells on surfaces even in samples negative for culture, with one study revealing viable cells in 76.7% of these samples. This situation shows that a significant amount of potentially useful biomass is overlooked with traditional approaches, while modern assessments consider cells that maintain their membrane structure and show some metabolic activity or response as alive, regardless of their inability to colonize on agar plates[47].

Ensuring Storage Stability

Storage stability involves the use of specific facilities, such as stability chambers and entry chambers, designed to preserve and assess how environmental factors affect product stability over time. It requires the control of specific environmental elements, including temperature, humidity, light levels, and other critical conditions necessary for evaluating shelf life, expiration dates, and optimal storage guidelines. Regulatory bodies such as the FDA in the US and the EMA in Europe oversee stability reviews to verify that products remain safe and effective throughout their designated storage periods and uses.

Dosage Standardization

Drugs and (LBPs) present significant challenges in standardizing dosages due to their complex biological structures, variability, and sensitivity to environmental influences. Authorities that regulating the dosages of live biotherapeutic products is one of the most important scientific and regulatory challenges. Stable storage plays a crucial role in meeting the necessary global regulatory standards to ensure product safety and protect consumer health. These reviews are essential to protect public health, enhance scientific credibility, and ensure confidence in product functionality. It adheres to a comprehensive methodology (Sensitive Stability Storage) that is frequently guided by the International Conference on Harmonization (ICH) [48].

Biosafety Concerns

Biosafety refers to the safe management of living organisms and genetic material, including pathogens and genetically modified organisms. According to international environmental law and policy, biosafety refers to the need to protect the environment and human health from the potential adverse effects of genetically modified organisms and products derived from modern biotechnology. Biosafety encompasses the establishment of protective measures, policies, and procedures to prevent accidental release or exposure to hazardous biological agents and to mitigate potential adverse effects arising from the use of modern biotechnology. Most importantly, it operates within a Risk Assessment and Risk Management framework that involves identifying potential hazards, assessing their probability and severity, and implementing appropriate control measures. The implementation of biosafety at the international level is primarily governed by the Cartagena Protocol on Biosafety to the Convention on Biological Diversity, which aims to ensure the safe handling, transport, and use of Living Modified

Organisms derived from modern biotechnology that may have adverse effects on biodiversity; This protocol also takes into account the risks to human health [49].

Potential Risks of Horizontal Gene Transfer Between Microorganisms

Horizontal Gene Transfer (HGT) refers to the transfer of genetic information between organisms through non-reproductive pathways. Unlike vertical gene transfer from parents to offspring, horizontal gene transfer allows microorganisms to rapidly acquire new traits, even from potentially unrelated species. While this phenomenon plays a significant role in the evolution and adaptation of microorganisms, its occurrence among these organisms poses several important risks, particularly in the fields of medicine and the environment [4, 23].

ADVANTAGES OF LIVE BIOTHERAPEUTIC PRODUCTS

Live Biotherapeutic Products (LBPs) present numerous important benefits compared to conventional therapies by employing biological and ecological processes that substantially lessen the selective pressure that contributes to antimicrobial resistance [1]. These items utilize various methods to block pathogens, including the competition for nutrients and binding locations to hinder mucosal settlement, as well as generating strong antimicrobials like bacteriocins that affect bacterial membranes [26], and disrupting biofilms to increase pathogen vulnerability [19]. Additionally, LBPs promote the modification of the host's immune response by engaging with immune cells, leading to the production of anti-inflammatory cytokines. This process improves the body's inherent ability to eliminate infections without relying on pharmaceutical treatments [39]. Cutting-edge biotechnology facilitates the exact and focused administration of therapeutic compounds right at the affected area, such as the gastrointestinal tract, while concurrently revitalizing the native human microbiome to uphold a balanced and healthy population of microorganisms [31].

FUTURE PERSPECTIVES

The prospects for Live Biotherapeutic Products, known as LBPs, are set to become a crucial aspect of worldwide health initiatives and the forthcoming generation of antimicrobial treatments thanks to ongoing developments in biotechnological and artificial intelligence fields. With the advancement of precision medicine, the use of metagenomic studies and bioinformatics will facilitate the identification of the strongest and most effective strains tailored to specific microbial compositions of individuals. In addition, the use of synthetic biology along with genetic modification will likely transform this domain by allowing the creation of "intelligent" microorganisms that are designed with genetic "kill switches" to enhance safety and ensure that therapies are delivered precisely at the site of illness. New patterns indicate that upcoming LBPs will adopt comprehensive strategies, such as merging living microorganisms with innovative encapsulation methods to enhance their survival throughout the gastrointestinal system and optimize therapeutic efficacy. Ultimately, as regulatory guidelines become increasingly uniform, LBPs are anticipated to shift from being considered alternative

solutions to becoming established biological medicinal products, successfully addressing the worldwide challenge of antimicrobial resistance while helping to restore the balance of human microbiota.

CONCLUSIONS

The findings presented in this article demonstrate that LBPs have been successfully developed and directed for medical use in the treatment and prevention of a wide variety of diseases and clinical conditions. LBPs are regulated as biological medicinal products and, unlike traditional probiotics and nutritional supplements, are designed to exhibit specific therapeutic effects.LBPs have shown significant clinical potential in various medical fields. One of the most established applications is the treatment and prevention of *Clostridioides difficile* infection, where LBPs contribute to restoring gut microbial balance and reducing disease recurrence. Additionally, LBPs have been investigated for the management of colitis and inflammatory bowel diseases, demonstrating the ability to modulate intestinal inflammation and improve mucosal health. Further findings highlight the emerging role of LBPs in cancer-related applications, particularly in cancer prevention strategies. Some live biotherapy products stimulate the production of beneficial metabolites and bioactive molecules that can support immune surveillance and inhibit pathways that promote tumor development. Live biotherapy products have been investigated as a therapeutic option for metabolic disorders such as phenylketonuria, as genetically engineered strains can metabolize toxic compounds and reduce disease burden. In particular, experimental evidence from studies on human colon explants has shown that the single-strain live biotherapy product *Bacillus velezensis* ADS024 can secrete proteases that reduce *C. difficile* toxin levels. This activity has been associated with protection against colon mucosal damage and a reduced risk of recurrent *C. difficile* infection following standard antibiotic treatment.

Several representative examples of live biotherapy products discussed in this article include *Escherichia coli* Nissle 1917, *Lactococcus lactis*, and *Salmonella typhi* VXM01; all of which demonstrate the therapeutic diversity and clinical significance of LBPs.

REFERENCES

[1] World Health Organization. (2021): *Antimicrobial resistance*. World Health Organization. Retrieved from https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance.

[2] Murray, C. J., Ikuta, K. S., Sharara, F., Swetschinski, L., Aguilar, G. R., Gray, A., et al. (2022): Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. *The Lancet*, 399(10325), 629–655. https://doi.org/10.1016/S0140-6736(21)02724-0.

[3] Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D. L., et al. (2022): Discovery, research, and development of new antibiotics: The WHO priority list of antibiotic-resistant bacteria and tuberculosis. *The Lancet Infectious Diseases*, 22(1), 142–150. https://doi.org/10.1016/S1473-3099(21)00457-X.

[4] Von Wintersdorff, C. J., Penders, J., van Niekerk, J. M., Mills, N. D., Majumder, S., van Alphen, L. B., et al. (2016): Dissemination of antimicrobial resistance in microbial

ecosystems through horizontal gene transfer. *Frontiers in Microbiology*, 7, 173. https://doi.org/10.3389/fmicb.2016.00173.

[5] National Institute of Allergy and Infectious Diseases (NIAID). (2021): *Antimicrobial resistance: Causes and mechanisms*. National Institutes of Health.

[6] Schuster, S., Vavra, M., & Kern, W. V. (2020): Evidence of a role of the multidrug efflux pump AcrAB-TolC in resistance to chlorhexidine in *Escherichia coli*. *Journal of Antimicrobial Chemotherapy*, 75(10), 2754–2762. https://doi.org/10.1093/jac/dkaa248.

[7] Blair, J. M., Webber, M. A., Baylay, A. J., Ogbolu, D. O., & Piddock, L. J. (2015): Molecular mechanisms of antibiotic resistance. *Nature Reviews Microbiology*, 13(1), 42–51. https://doi.org/10.1038/nrmicro3380.

[8] Li, X. Z., Plésiat, P., & Nikaido, H. (2021): The challenge of efflux-mediated antibiotic resistance in Gram-negative bacteria. *Clinical Microbiology Reviews*, 34(1). https://doi.org/10.1128/CMR.00031-20.

[9] Høiby, N., Ciofu, O., & Bjarnsholt, T. (2020): *Pseudomonas aeruginosa* biofilms in cystic fibrosis. *Future Microbiology*, 15(1), 25–31. https://doi.org/10.2217/fmb-2019-0111.

[10] Schuster, S., Vavra, M., & Kern, W. V. (2020): Evidence of a role of the multidrug efflux pump AcrAB-TolC in resistance to chlorhexidine in *Escherichia coli*. *Journal of Antimicrobial Chemotherapy*, 75(10), 2754–2762. https://doi.org/10.1093/jac/dkaa248.

[11] Li, X. Z., Plésiat, P., & Nikaido, H. (2022): Efflux-mediated drug resistance in bacteria: An update. *Drugs*, 82(13), 1529–1561. https://doi.org/10.1007/s40265-022-01772-x.

[12] Kyrikou, I., & Briassoulis, D. (2007): Biodegradation of agricultural plastic films: A critical review. *Journal of Polymers and the Environment*, 15, 227–241. https://doi.org/10.1007/s10924-007-0063-6.

[13] Shah, A. A., Hasan, F., Hameed, A., & Ahmed, S. (2008): Biological degradation of plastics: A comprehensive review. *Biotechnology Advances*, 26, 246–265.

[14] Chen, R., Ma, C., Liu, Z., Bian, R., Liu, H., & Zhou, X. (2020): Enzymatic degradation of plant biomass and synthetic polymers. *Acta Polymerica Sinica*, 1(1), 1–13.

[15] Emadian, S. M., Onay, T. T., & Demirel, B. (2017): Biodegradation of bioplastics in natural environments. *Waste Management*, 59, 526–536.

[16] Tosin, M., Naimi, Y., & Degli-Innocenti, F. (2019): Biodegradation kinetics in soil of a multi-constituent biodegradable plastic. *Polymer Degradation and Stability*, 164, 149–157.

[17] Bátori, R., Wirth, R., Forgács, G., Orosz, V., Kovács, B., & Gergely, S. (2018): Anaerobic degradation of bioplastics: A review. *Journal of Waste Management*, 80, 50–61.

[18] Stewart, P. S. (2002): Mechanisms of antibiotic resistance in bacterial biofilms. *International Journal of Medical Microbiology*, 292(2), 107–113. https://doi.org/10.1078/1438-4221-00196.

[19] Costerton, J. W., Stewart, P. S., & Greenberg, E. P. (1999): Bacterial biofilms: A common cause of persistent infections. *Science*, 284(5418), 1318–1322. https://doi.org/10.1126/science.284.5418.1318.

[20] Kato, H., Yamada, K., Tada, T., et al. (2020): Penicillin-binding protein 2a is an important factor for the survival of methicillin-resistant *Staphylococcus aureus* in the presence of β-lactam antibiotics. *Journal of Infection and Chemotherapy*, 26(7), 723–728. https://doi.org/10.1016/j.jiac.2019.12.013.

[21] Diene, S. M., & Rolain, J. M. (2014): Mechanisms of bacterial resistance: Focus on efflux pumps, drug permeability, and target modification. *Future Microbiology*, 9(10), 1279–1293. https://doi.org/10.2217/fmb.14.91.

[22] Delcour, A. H. (2009): Outer membrane permeability and antibiotic resistance. *Biochimica et Biophysica Acta*, 1794(5), 808–816. https://doi.org/10.1016/j.bbapap.2008.11.005.

[23] Perry, J. A., & Wright, G. D. (2013): The antibiotic resistance mobilome: Searching for the link between environment and clinic. *Frontiers in Microbiology*, 4, 138. https://doi.org/10.3389/fmicb.2013.00138.

[24] Food and Drug Administration. (2016): *Early clinical trials with live biotherapeutic products: Chemistry, manufacturing, and control information. Guidance for Industry*. U.S. Food and Drug Administration.

[25] Ismael, M., Huang, M., & Zhong, Q. (2024): The bacteriocins produced by lactic acid bacteria and their promising applications in promoting gastrointestinal health. *Foods*.

[26] Cotter, P. D., Ross, R. P., & Hill, C. (2005): Bacteriocins—A viable alternative to antibiotics? *Nature Reviews Microbiology*, 3(10), 777–789.

[27] Breukink, E., & de Kruijff, B. (1999): The mechanism of lipid II inhibition by nisin. *Current Opinion in Chemical Biology*, 3(4), 367–372.

[28] Ghosh, A., Loper, J. E., & Thomashow, L. S. (2019): Production of bacteriocins and their role in biofilm inhibition. *Frontiers in Microbiology*, 10, 1630.

[29] Chang, M. F., Kistler, W., Jarratt, N., Abali, E., Styczynski, M., & Hammerling, M. J. (2022): Discovery and delivery strategies for engineered live biotherapeutic products. *Trends in Biotechnology*, 40(3), 354–369. https://doi.org/10.1016/j.tibtech.2021.08.002.

[30] Endy, D. (2005): Foundations for engineering biology. *Nature*, 438(7067), 449–453.

[31] Mimee, M., Lu, T. K., & Wanner, B. L. (2018): Synthetic biology approaches for engineering therapeutic microbes. *Trends in Biotechnology*, 36(6), 542–555.

[32] National Human Genome Research Institute (NHGRI). (n.d.): *Metagenomics*. Genome.gov. Retrieved from https://www.genome.gov/genetics-glossary/Metagenomics.

[33] Lee, B., Jung, J., & Kim, Y. (2021): Metagenomic approaches for understanding and engineering microbial communities in the gut. *Trends in Microbiology*, 29(12), 1121–1132.

[34] Paquet, D., et al. (2024): Navigating regulatory and analytical challenges in live biotherapeutic product development and manufacturing. *Frontiers in Microbiomes*.

[35] Jan, T., Negi, R., Hilal, A., Kaur, S., Kour, D., Khan, S. S., Shreaz, S., Chauhan, P., Yadav, N., Rustagi, S., Rai, A. K., Singh, S., Yadav, A. N., & Ahmed, N. (2025): Microencapsulation techniques for probiotic formulations: Current scenario and future perspective. *Journal of Food Quality*, 2025, Article 6738124. https://doi.org/10.1155/jfq/6738124.

[36] Bikard, D., & Barrangou, R. (2017): Using CRISPR-Cas systems as antimicrobials. *Current Opinion in Microbiology*, 37, 155–160. https://doi.org/10.1016/j.mib.2017.08.005.

[37] Geller, R., et al. (2017): CRISPR-Cas9 as an antiviral and antimicrobial tool. *Current Opinion in Virology*, 26, 142–148.

[38] Microbiome Therapeutics Innovation Group, & Barberio, D. (2024): Navigating regulatory and analytical challenges in live biotherapeutic product development and manufacturing. *Frontiers in Microbiology*.

[39] Zhang, Y., Zhou, L., Xia, J., Dong, C., & Luo, X. (2021): Human microbiome and its medical applications. *Frontiers in Molecular Biosciences*, 8. https://doi.org/10.3389/fmolb.2021.703.

[40] Kwon, M. S., & Lee, H. K. (2022): Host and microbiome interplay shapes the vaginal microenvironment. *Frontiers in Immunology*, 13. https://doi.org/10.3389/fimmu.2022.919728.

[41] Heavey, M. K., Durmusoglu, D., Crook, N., & Anselmo, A. C. (2022): Discovery and delivery strategies for engineered live biotherapeutic products. *Trends in Biotechnology*, 40, 354–369. https://doi.org/10.1016/j.tibtech.2021.08.002.

[42] Tan, Y., Shen, J., Si, T., Ho, C. L., Li, Y., & Dai, L. (2020): Engineered live biotherapeutics: Progress and challenges. *Biotechnology Journal*, 15, e2000155. https://doi.org/10.1002/biot.202000155.

[43] O'Donnell, M. M., Hegarty, J. W., Healy, B., Schulz, S., Walsh, C. J., Hill, C., et al. (2022): Identification of ADS024, a newly characterized strain of *Bacillus velezensis* with direct *Clostridioides difficile* killing and toxin degradation bioactivities. *Scientific Reports*, 12, 9283. https://doi.org/10.1038/s41598-022-13248-4.

[44] Borman, P., & Al-Tameemi, M. (2020): Regulatory considerations for live biotherapeutic products: A global perspective. *Pharmaceutical Technology*, 44(10), 20–25.

[45] Food and Drug Administration (FDA). (2008): *Guidance for Industry: CGMP for Phase 1 Investigational Drugs*. U.S. Food and Drug Administration.

[46] Food and Drug Administration, Center for Drug Evaluation and Research (FDA CDER). (2003): *Guidance for Industry: INDs for Phase 2 and Phase 3 Studies—Chemistry, Manufacturing, and Controls Information*. U.S. Food and Drug Administration.

[47] Nocker, A., Suller, M. T. E., & Burgener, C. L. (2022): Viability assessment of microorganisms using nucleic acid dyes and flow cytometry: An update on methods, challenges and emerging applications. *Journal of Microbiological Methods*, 200, 106579.

[48] International Council for Harmonisation (ICH). (2003): *ICH Q1A (R2): Stability testing of new drug substances and drug products*. European Medicines Agency.

[49] Rouanet, A., Bolca, S., Bru, A., Claes, I., Cvejic, H., Girgis, H., ... & Cordaillat-Simmons, M. (2020). Live biotherapeutic products, a road map for safety assessment. Frontiers in Medicine, 7, 237.