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Biorisks Associated with Synthetic Biology: Virulence plasmid Transfer to Probiotic or Starter Cultures

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ABSTRACT
Synthetic biology holds promise for revolutionizing biotechnology across diverse fields, yet it also presents inherent biorisks that require careful consideration and mitigation strategies. This paper explores the potential biorisks associated with the transfer of virulence plasmids to probiotic or starter cultures within synthetic biology applications. Beginning with an introduction to synthetic biology principles and the significance of probiotics and starter cultures, the discussion delineates the mechanisms and consequences of virulence plasmid transfer, drawing insights from case studies involving bacterial species like Bifidobacterium and Bacillus, as well as yeast probiotics such as Saccharomyces. Notably, the incidents underscore the emergence of antibiotic resistance genes and multidrug resistance, emphasizing the critical need for robust containment measures and genetic safety precautions. Ethical considerations permeate the discourse, advocating for transparency, informed consent, and responsible innovation in synthetic biology. Ultimately, this paper underscores the importance of global collaboration, harmonized regulatory frameworks, and continual evaluation to ensure the safe and ethical utilization of synthetic biology techniques, thus maximizing benefits while minimizing potential risks to human health and the environment.

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Introduction

Synthetic biology is a rapidly advancing multidisciplinary field that integrates principles from biology, engineering, and computer science to design and construct novel biological systems [1]. The ability to manipulate and engineer biological organisms has led to a wide range of applications, including the development of improved therapeutics, sustainable biofuels, and enhanced agricultural products [2,3,4]. While the potential benefits of synthetic biology are undeniable, it is essential to recognize and address the potential biorisks associated with these technological advancements [5-9].

One significant biorisk in the context of synthetic biology is the transfer of virulence plasmids to probiotic or starter cultures. Probiotics, which consist of beneficial live microorganisms, are consumed to confer health benefits to the host [10]. Similarly, starter cultures are used in various fermentation processes to initiate specific biochemical reactions [11]. The accidental introduction of a virulence plasmids into these modified organisms may result in unintended consequences, leading to enhanced pathogenicity, potential human health risks, and ecological ramifications [12, 13].

It is essential to comprehend and effectively handle the biorisks linked to synthetic biology methods to fully unlock the potential of this field, all while guaranteeing the safety of both human users and the environment. By identifying potential risks and implementing robust risk mitigation strategies, we can foster responsible innovation and safeguard against unintended consequences in the use of synthetic biology for probiotics and starter cultures. The first section will provide a brief overview of synthetic biology, highlighting its key principles and applications, with a specific focus on the engineering of probiotics and starter cultures. The subsequent sections will delve into the risks associated with virulence plasmid transfer, the potential consequences of such transfers, and strategies to mitigate these risks. Furthermore, case studies of previous incidents involving plasmid transfers in synthetic biology applications will be presented, offering insights into lessons learned and their implications for future research. Ethical considerations and the importance of

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effective communication with the public will also be addressed, recognizing the need to strike a balance between the benefits and potential risks of synthetic biology.

This paper aims to examine the potential biorisks associated with the transfer of virulence plasmids to probiotic or starter cultures. It will explore the mechanisms by which such transfers might occur, analyze the consequences and impacts of such events, and propose risk mitigation strategies to ensure the responsible and safe use of synthetic biology in these applications.

Background

Synthetic biology and genetic engineering

Synthetic biology has revolutionized biotechnology, offering new ways to design and construct biological systems with precision and efficiency. It involves the deliberate design of genetic circuits, metabolic pathways, and other biological components to achieve specific functions [14]. Techniques like gene synthesis [15], gene editing (e.g., CRISPR-Cas9) [16, 17], and DNA assembly [18] enable scientists to engineer living organisms with desired traits and capabilities. Genetic engineering is a fundamental aspect of synthetic biology, allowing researchers to manipulate an organism's genetic material [19]. This involves inserting or deleting specific genes or genetic elements to confer desired properties, such as disease resistance or improved product synthesis, to microorganisms [20, 21].

Probiotics and starter cultures

Probiotics are live microorganisms, including bacteria and yeasts, consumed as dietary supplements or added to food products for potential health benefits [9]. These beneficial microbes aim to restore or enhance the balance of gut bacteria, promoting better digestion, immune function, and overall well-being [9]. Probiotics have gained popularity among consumers seeking natural approaches to support gut health [22-24].

Starter cultures, on the other hand, play a crucial role in food and beverage fermentation processes [11, 25]. These microorganisms initiate controlled biochemical reactions, transforming raw ingredients into various products, such as yogurt, cheese, bread, sauerkraut, and alcoholic beverages [26]. Specific starter cultures are chosen for their ability to produce particular enzymes or metabolic products, influencing the final product's taste, texture, and safety [11].

Virulence plasmids and horizontal gene transfer (HGT)

Virulence plasmids are small, circular DNA elements commonly found in pathogenic bacteria [27]. They carry genes responsible for pathogenicity, antibiotic resistance, and other virulence factors. Horizontal Gene Transfer (HGT) is a process where bacteria transfer genetic material, including plasmids, to other microorganisms [28]. HGT occurs through mechanisms like conjugation, where plasmids are directly transferred between bacteria, transduction, where bacteriophages (viruses infecting bacteria) mediate the transfer of genetic material, and transformation, where bacteria take up foreign DNA from their surroundings [29]. When virulence plasmids are transferred to probiotic or starter cultures through HGT, these normally benign microorganisms may acquire harmful genes and traits. This unintended acquisition could lead to the expression of dangerous proteins, compromising the safety and health benefits of these modified organisms [30-32].

The potential biorisks associated with virulence plasmid transfer to probiotic or starter cultures will be explored and discussing the potential consequences and risk mitigation strategies to ensure the responsible use of synthetic biology techniques in these applications will be made.

Biorisks of Virulence plasmid Transfer

The transfer of virulence plasmids to probiotic or starter cultures presents significant biorisks that demand careful consideration and risk mitigation strategies. These risks primarily arise from the potential for horizontal gene transfer (HGT) of virulence genes and pathogenic traits, leading to unintended consequences and posing threats to human health and the environment [33-35]. This section will delve into the specific biorisks associated with virulence plasmid transfer in the context of synthetic biology applications.

Horizontal gene transfer (HGT)

While HGT plays a crucial role in bacterial evolution and adaptation, it becomes a cause for concern when virulence plasmids carrying harmful genetic elements are inadvertently transferred to probiotic or starter cultures. Unlike vertical gene transfer, where genes are passed from parent to offspring [36], HGT enables the rapid dissemination of genetic information across diverse microbial populations [37-40].

In the case of probiotics, HGT of virulence plasmids could potentially transform harmless strains into virulent pathogens. This transformation may result from the expression of virulence factors or antibiotic resistance genes present in the transferred plasmids [41-43]. Similarly, starter cultures that acquire plasmids from pathogens may produce harmful byproducts during food fermentation, compromising food safety and quality [44, 45].

Enhanced pathogenicity

The acquisition of virulence plasmids by probiotic or starter cultures can confer new pathogenic traits or enhance existing ones [46, 47]. Modified microorganisms may gain the ability to produce toxins, evade the host's immune system, or colonize host tissues more effectively [48]. In the context of starter cultures, enhanced pathogenicity may lead to the production of contaminated food products, posing risks to consumers' health [49, 50].

Environmental spread

The release of genetically modified probiotics or starter cultures into the environment, whether intentional or unintentional, can introduce virulence plasmids into ecosystems and other microbial populations. Environmental spread may occur through interactions with other microorganisms, dissemination through waste streams, or survival in soil and water environments [51]. The potential for environmental spread poses broader ecological risks, as virulence plasmids may transfer to other bacteria in the environment. If these transferred plasmids carry antibiotic resistance genes, they could contribute to the development of antibiotic-resistant bacteria, exacerbating the global health crisis of antimicrobial resistance (AMR) [52 - 54]. Next, the potential consequences and impacts of virulence plasmid transfer to probiotic or starter cultures will be discussed, followed by risk mitigation strategies to address these biorisks responsibly and ensure the safe utilization of synthetic biology in these applications.

Consequences And Impact

Understanding these potential outcomes is crucial for developing effective risk mitigation strategies and ensuring responsible applications of synthetic biology in the context of probiotics and starter cultures.

Health risks to human consumers

If probiotics carrying virulence plasmids reach consumers, they may inadvertently introduce harmful traits into the gut microbiota, leading to gastrointestinal infections or exacerbating existing health conditions. Moreover, individuals with compromised immune systems, such as the elderly, young children, and those undergoing medical treatments, may be more susceptible to the adverse effects of ingesting pathogenic probiotics. While lactic acid bacteria are commonly considered safe and typically do not induce illness, sporadic instances of infections have been documented in individuals undergoing antibiotic therapy or experiencing severe immunocompromise [55, 56]. In such cases, conditions such as endocarditis, bacteremia, and localized infections such as abdominal abscesses, pulmonary infections, and peritonitis are among the most frequently reported diseases associated with Lactobacillus spp [57]. In the human digestive tract, enterococci—mostly E. faecalis and E. faecium—are common microorganisms. These species are frequently isolated from human feces, along with Enterococcus durans [58]. Even while enterococci are commonly found in food products, can be used as starter cultures, and may even be advantageous probiotics, they are opportunistic pathogens that frequently cause nosocomial infections. They are intrinsically resistant to low concentrations of many antibiotic drugs, such as tetracycline, aminoglycosides, clindamycin, lincomycin, and beta-lactams [58, 59]. Enterococci have been found to possess a variety of virulence factors [60, 61]. This heightened vulnerability highlights the need for stringent risk assessment and containment measures to prevent the accidental release of modified organisms into the market.

Contamination of food and fermentation processes

The contamination of starter cultures with virulence plasmids can lead to the production of contaminated food items, posing significant risks to consumers. Consumption of such products could result in foodborne illnesses and outbreaks, leading to economic losses for the food industry and public health concerns [61-63].

In addition to direct contamination, the presence of virulence plasmids in starter cultures may alter fermentation processes, affecting product quality and consistency. The unintended expression of pathogenic traits by modified starter cultures could lead to unwanted byproducts, spoilage, or changes in taste and texture [64].

Environmental dissemination and ecological consequences

The escape or intentional release of modified probiotics or starter cultures into the environment raises ecological concerns. If virulence plasmids spread to other microorganisms in natural ecosystems, it could lead to the unintended development of pathogenic strains in environmental niches [65, 66]. This environmental spread may also contribute to the dissemination of antibiotic resistance genes [65, 67], further aggravating the global issue of AMR and compromising the effectiveness of antibiotics in medicine and agriculture. Moreover, the ecological consequences of uncontrolled dissemination are challenging to predict fully. The introduction of genetically modified microorganisms into ecosystems may disrupt existing ecological balances and interactions, potentially leading to unforeseen ecological disturbances and imbalances in microbial communities [68-71].

In the following section, essential risk mitigation strategies will be discussed to address the biorisks associated with virulence plasmid transfer to probiotic or starter cultures. The aim of these strategies is to ensure the responsible and safe use of synthetic biology techniques in these applications while maximizing their potential benefits. Additionally, valuable insights and lessons for future research and risk management will be provided through case studies of previous incidents involving plasmid transfers.

Risk Mitigation Strategies

To address the biorisks associated with the transfer of virulence plasmids to probiotic or starter cultures, a proactive approach to risk mitigation is essential. Employing a combination of stringent containment measures, genetic safety precautions, and comprehensive risk assessments can help ensure the responsible use of synthetic biology techniques in these applications [72, 73].

Containment measures

Stringent containment protocols are critical to prevent the accidental release of modified microorganisms into the environment. These measures involve physical containment and strict adherence to standard operating procedures to minimize the risk of escape or unintentional dissemination [74, 75]. Laboratories and facilities involved in synthetic biology research should follow appropriate biosafety guidelines and operate under the principles of the "containment hierarchy," which involves multiple layers of protection to prevent exposure to hazardous microorganisms [76].

In addition to laboratory containment, the commercial use of genetically modified probiotics or starter cultures should adhere to robust containment measures [77]. This may include the use of closed fermentation systems [78], a controlled environment, and secure waste disposal procedures to prevent the spread of virulence plasmids to the external environment [79, 80].

Robust genetic safety measures

Genetic safety measures can be employed to reduce the likelihood of plasmid transfer and mitigate potential risks associated with virulence plasmids. One such approach is the use of "suicide genes" or "genetic kill switches," which are genetic elements that cause the death or self-destruction of the modified microorganism under specific conditions [51, 81]. Incorporating these safety mechanisms can ensure that the modified organisms have limited survival and proliferation outside controlled environments, providing an added layer of biocontainment [82,83].

Another genetic safety strategy involves the use of "gene containment systems" to limit horizontal gene transfer [84 - 86]. These systems prevent the transfer of engineered genetic elements, such as plasmids, to other microorganisms [84 - 86]. For instance, synthetic biology researchers can design plasmids with built-in safeguards that restrict their transferability or limit their stability outside of the target organism [84].

Comprehensive risk assessment

Before the release or commercialization of any synthetic biology application involving modified probiotics or starter cultures, comprehensive risk assessments must be conducted. Risk assessment processes should evaluate potential biorisks, consider the intended use, and assess the likelihood and severity of unintended consequences [87, 88].

Risk assessment should be an ongoing and iterative process, continually reevaluating and updating strategies as new scientific information and data become available [89]. This dynamic approach ensures that the most current knowledge and best practices are applied to minimize risks effectively.

In the next section, we will explore relevant case studies of previous incidents involving plasmid transfers in synthetic biology applications. By examining these incidents, we can learn from past experiences and identify areas for improvement in risk management and safety practices.

Case Studies

Examining past incidents involving plasmid transfers in synthetic biology applications provides valuable insights into the potential risks and challenges associated with these technologies. Learning from these case studies can help inform future research, risk management, and the development of improved safety measures.

Incidents of plasmid transfer in synthetic biology applications

Conjugation, phage-associated transduction, and transformation all help to reduce mutant genome transfer between bacterial species via integrons or transposons [90, 91]. Human studies have shown that the incidence of HGT is substantially higher in humans, particularly among gut microbiota and ingested probiotics, implying that they serve as reservoirs of resistance genes. Furthermore, resistance in the oral cavity microbiota, particularly among *Streptococci*, and resistance to tetracycline caused by genes such as tet(M), tet(O), tet(Q), and tet(W), are significant examples of AMR [92].

In Bifidobacterium species, the transfer of genetic elements, especially antibiotic resistance genes, is commonly observed. While intrinsic resistance to mupirocin is prevalent among most species due to its role in protein synthesis, resistance to streptomycin can arise from mutations in the rpsL gene in *B.bifidum* and *B.breve*. Some studies suggest that streptomycin resistance may result from chromosomal mutations, rendering it non-transferable [93, 94]. Several tetracycline resistance-associated genes in species of *Bifidobacterium* have been well-characterized, with *B. adolescentis* and *B. longum* demonstrating the transfer of the tet(W) gene under in vitro conditions. [95], though in-vivo transferability warrants further investigation. Similarly to the *Lactobacillus genus, B. breve* and *B. longum* harbor transporters capable of translocating unrelated compounds, leading to multidrug resistance (MDR) [96]. Certain resistance genes identified in other probiotic species, such as *Lactococcus lactis*, can be transferred to other bacteria, exemplified by tetracycline resistance genes being transferred to *Enterococcus faecalis. Bacillus spp*., routinely employed as probiotics, frequently produce toxins, and while some strains lack plasmids harboring resistance genes, others might transfer AMR genes, omitting resistance to ampicillin [97]. Extrachromosomal elements contribute to resistance to macrolide and tetracycline antibiotics among *Bacillus spp*., with studies focusing on resistance induced by the transfer of CFR-related genes, which encode ribosomal methyltransferase, to other bacteria in the intestine. Safety assessments of Bacillus spp. are currently inadequate and necessitate further investigation [98].

In contrast to bacterial strains, research on the AMR of yeast probiotics is limited. However, a study comparing antimicrobial resistance between bacteria and yeasts in a commercial probiotic product found that *Saccharomyces faecalis* and *Saccharomyces boulardii* exhibited resistance to 80% of antibiotics tested [99]. Additionally, *Pichia kudriavzevii* demonstrated intrinsic resistance to benzylpenicillin and vancomycin, suggesting potential transferability between microorganisms [100].

Lessons learned and implications for future research.

Ensuring the safe and responsible use of synthetic biology techniques is of utmost importance, especially when it comes to dealing with potential biorisks like virulence plasmid transfer to probiotic or starter cultures. To achieve this, several crucial aspects need to be considered. Firstly, understanding the genetic makeup of genetically modified microorganisms is vital. Researchers must thoroughly analyze the plasmids and other genetic elements introduced into these organisms to identify any potentially harmful genes or traits [101]. Secondly, comprehensive risk assessment should be an integral part of the entire research and development process. This should start right from the design phase and continue through the application and potential release stages [102]. By identifying potential risks early on, appropriate risk mitigation strategies can be put in place. Implementing biocontainment strategies and genetic safety measures is another crucial step. Robust containment measures and safety precautions can limit the spread of modified organisms, reducing the risk of unintended plasmid transfer. Moreover, incorporating "suicide genes" or "genetic kill switches" can ensure that these organisms have limited survival outside controlled environments, adding an extra layer of safety [50, 80]. Transparency and effective communication with stakeholders are equally important. This includes engaging with the public and regulatory authorities to address concerns and foster a shared understanding of the potential risks and benefits of synthetic biology applications. Post-market surveillance is essential to continuously monitor the behavior of genetically modified microorganisms after commercial release [103]. This allows for the identification of any unforeseen outcomes or evolving risks, enabling timely interventions and updates to safety measures. Emphasizing the principle of precaution is central to ethical decision-making [103]. Even in the absence of complete scientific certainty, taking precautionary measures ensures that potential risks are thoroughly assessed and prioritized. Global collaboration between scientists, policymakers, and regulatory bodies is critical. Working together can lead to harmonized regulatory standards and ethical frameworks, ensuring responsible innovation and safeguarding against biorisks across borders [104]. Public engagement and involvement in decision-making processes are also vital. Including a diverse group of stakeholders in ethical review boards and public consultations helps gather valuable insights and ensures that public concerns and preferences are taken into account when shaping regulations and policies [105]. It should be taken into account that adopting a holistic approach that encompasses genetic characterization, risk assessment, biocontainment strategies, transparent communication, and ethical considerations is key to addressing biorisks associated with synthetic biology applications. By embracing responsible practices, monitoring developments closely, and fostering global collaboration, we can unlock the full potential of synthetic biology while ensuring the safety and well-being of both humans and the environment. These case studies underscore the importance of continual evaluation, collaboration between researchers and policymakers, and a proactive approach to biosecurity and risk management in synthetic biology.

Implications for responsible innovation

Synthetic biology has the potential to revolutionize various industries and contribute to significant advancements in biotechnology. However, ensuring responsible innovation is paramount to prevent

unintended negative consequences. Researchers, policymakers, and stakeholders must work together to strike a balance between embracing the transformative potential of synthetic biology and safeguarding against potential risks.

The ethical considerations surrounding synthetic biology applications involving virulence plasmid transfer will be explored in the following section. Ethical analysis is used to guide decision-making, encourage transparency, and ensure that the development and deployment of these technologies address societal values and concerns.

Ethical Considerations

The responsible development and deployment of synthetic biology applications involving virulence plasmid transfer to probiotic, or starter cultures demand careful ethical scrutiny. Ethical considerations play a pivotal role in guiding decision-making, ensuring the protection of human health, the environment, and societal values. This section explores key ethical considerations associated with these technologies.

Balancing benefits and risks in synthetic biology

As with any technological advancement, synthetic biology offers immense potential benefits, such as improved healthcare, sustainable agriculture, and environmental remediation [106]. However, it is essential to weigh these potential benefits against the associated risks carefully. Ethical decision-making requires a comprehensive analysis of the potential harms and benefits, taking into account both short-term and long-term consequences.

Balancing the risks and benefits of synthetic biology applications involving virulence plasmid transfer requires transparency, scientific evidence, and public engagement [107]. Policymakers, researchers, and industry stakeholders must involve the public in discussions about the applications' potential implications, fostering open dialogue to ensure that societal values and ethical concerns are adequately addressed.

Public perception and communication

Public perception and understanding of synthetic biology are crucial for its acceptance and responsible implementation. Synthetic biology applications, especially those involving genetically modified microorganisms, can raise concerns among the public regarding safety, health, and environmental impacts. Transparent and effective communication about the risks, benefits, and risk mitigation strategies is essential to build public trust and address public concerns.

Engaging with the public through educational programs, public forums, and stakeholder consultations can help foster informed decision-making and ensure that the public's values and preferences are considered in the development and regulation of these technologies [108]. Ethical communication should strive to be accessible, accurate, and free from sensationalism, fostering a constructive and well-informed public debate [109].

Informed consent and responsible use

The ethical use of synthetic biology technologies demands the principle of informed consent. Individuals who may be affected by the applications should have access to clear and accurate information about the potential risks and benefits. In the context of probiotics or starter cultures, consumers should be informed if the product contains genetically modified microorganisms and understand any associated risks [110].

Responsible use also involves adherence to regulatory guidelines and ethical standards. Research institutions, industries, and policymakers must prioritize safety and ethical considerations, ensuring compliance with established biosafety protocols and regulations. This commitment to responsible practices should extend to the entire lifecycle of the technology, from research and development to commercialization and post-market monitoring [111].

Next, recommendations for regulations and policy frameworks will be presented to support the safe and responsible use of synthetic biology techniques involving virulence plasmid transfer to probiotic or starter cultures. These recommendations aim to foster responsible innovation while safeguarding against potential biorisks and ethical concerns.

Global collaboration and responsible innovation

Given the global nature of synthetic biology and the potential for its applications to cross borders, international collaboration is vital for responsible innovation. Global cooperation can facilitate the exchange of best practices, harmonization of regulatory standards, and collective efforts to address shared biosecurity concerns [112, 113].

Responsible innovation in synthetic biology requires a continuous commitment to improving safety measures, updating risk assessments, and addressing emerging challenges. By proactively integrating ethical considerations into research, development, and application, the field can move forward with the potential to benefit humanity while minimizing potential risks [114, 115].

Regulations and Policy Recommendations

To ensure the safe and responsible use of synthetic biology techniques involving virulence plasmid transfer to probiotic or starter cultures, a robust regulatory framework is essential. This section presents key policy recommendations and regulatory guidelines to address the potential biorisks and ethical considerations associated with these applications.

International guidelines for synthetic biology applications

Harmonization of regulations and guidelines at the international level is critical to foster responsible innovation and mitigate global biorisks. Collaborative efforts between governments, scientific organizations, and international bodies can establish uniform safety standards, risk assessment protocols, and ethical frameworks for synthetic biology applications. International guidelines should prioritize the principles of precaution and transparency. Risk assessments should be conducted for each synthetic biology application, with an emphasis on addressing uncertainties and potential unintended consequences [116]. These guidelines can also serve as a foundation for national and regional regulatory frameworks, providing a consistent approach to ensure safety and ethical considerations [117].

The Cartagena Protocol on Biosafety is a key international treaty addressing the safe handling of living modified organisms (LMOs) from modern biotechnology, including synthetic biology [118-120]. It establishes a framework for transboundary regulation to prevent adverse effects on biodiversity and human health during development, handling, transport, and trade [120]. Emphasizing risk assessment, it encourages nations to evaluate potential environmental and human health risks, fostering effective mitigation measures. The protocol promotes information exchange, transparency, and capacity building, particularly in developing countries [120].

Harmonization of biosafety standards

Within each country, harmonization of biosafety standards is essential to promote uniform safety practices and facilitate the international exchange of genetically modified microorganisms [121]. Regulatory agencies should work collaboratively to develop risk assessment methodologies, containment requirements, and reporting systems for synthetic biology applications. Adopting a risk-based approach can help tailor safety measures to the specific characteristics of each application. Researchers, industries, and regulators should actively engage in ongoing dialogue to identify emerging risks, assess their potential impacts, and update safety guidelines accordingly [122].

Encouraging collaboration between scientists and policymakers

Close collaboration between the scientific community and policymakers is vital to inform evidence-based regulations and ensure that policy decisions align with the latest scientific knowledge. Policymakers should actively seek input from scientists and experts in the field of synthetic biology to develop informed and adaptable regulations. Additionally, fostering interdisciplinary collaborations among scientists, engineers, ethicists, and social scientists can promote a holistic understanding of the potential impacts of synthetic biology applications [123]. This interdisciplinary approach enables the consideration of ethical, social, and environmental dimensions, leading to more comprehensive risk assessments and policy development.

Pre-market and post-market surveillance

Comprehensive pre-market risk assessments are essential for evaluating the safety of synthetic biology applications before commercial release. Risk assessments should include rigorous testing of engineered organisms, including their genetic stability, potential for horizontal gene transfer, and potential environmental impacts [116, 124]. Post-market surveillance is equally important to monitor the long-term safety and performance of synthetic biology applications. Tracking the behavior of modified microorganisms in real-world settings can help identify any unexpected outcomes or evolving risks, enabling timely intervention and necessary updates to safety measures [103].

Public engagement and ethical review

Public engagement should be an integral part of the decision-making process concerning synthetic biology applications. Ethical review boards, involving a diverse group of stakeholders, can assess the ethical implications of specific applications and provide guidance on responsible innovation and risk management.

Public consultations can provide valuable feedback on societal values, concerns, and expectations related to synthetic biology applications. This feedback should be taken into account in the development of regulations and policies to ensure alignment with public preferences and values.

In conclusion, robust regulations and policy frameworks are crucial for harnessing the transformative potential of synthetic biology while minimizing potential biorisks and ethical concerns. By promoting international collaboration, harmonizing biosafety standards, fostering interdisciplinary cooperation, and engaging the public, we can navigate the complexities of synthetic biology responsibly and ethically, ensuring a safer and more sustainable future for these innovative technologies.

Conclusion and Discussion

The rapid advancements in synthetic biology have unlocked vast possibilities for biotechnological innovation, with applications ranging from healthcare to agriculture. However, as we venture further into this promising field, it is imperative to approach its applications with a cautious and responsible mindset. The transfer of virulence plasmids to probiotic or starter cultures presents significant biorisks that demand careful consideration and risk mitigation. This paper has provided a comprehensive exploration of the biorisks associated with virulence plasmid transfer in the context of synthetic biology applications. The potential for horizontal gene transfer and enhanced pathogenicity underscores the need for robust containment measures and genetic safety precautions. Understanding the consequences of unintended plasmid transfer on human health, food safety, and the environment highlights the importance of comprehensive risk assessments at all stages of development and deployment. Ethical considerations play a central role in guiding responsible decision-making and policy development. Striking a balance between the benefits and risks of synthetic biology applications involves transparent communication, public engagement, and adherence to ethical principles such as informed consent and responsible use. To ensure the safe and ethical use of synthetic biology techniques, international collaboration is vital for harmonizing regulatory standards and sharing best practices. Policymakers, scientists, and stakeholders must work together to develop and implement robust regulatory frameworks that prioritize safety, accountability, and the public interest. As we move forward in the field of synthetic biology, we must remain vigilant, continually evaluating and improving safety measures, risk assessments, and ethical practices. Lessons learned from previous incidents and case studies serve as invaluable guides for future research and risk management. By embracing responsible innovation and adhering to the highest ethical standards, we can unlock the full potential of synthetic biology while safeguarding human health, protecting the environment, and respecting societal values. The responsible use of synthetic biology techniques involving virulence plasmid transfer to probiotic, or starter cultures will pave the way for a more sustainable, healthier, and safer future for humanity and our planet. By fostering a culture of responsible research and decision-making, we can harness the transformative power of synthetic biology to address pressing challenges and improve lives while minimizing potential risks and ensuring a more secure and sustainable future for generations to come.

Abbreviations

AMR: Antimicrobial resistance, HGT: Horizontal Gene Transfer, LMOs: Living modified organisms, MDR: Multidrug resistance

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Compliance with ethical standards

Conflict of interest / Çıkar çatışması

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Ethical standards

The study is proper with ethical standards.

Authors' contributions

All authors contributed equally to this work.

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