Potential Harmful Effects of Genetically Engineered Microorganisms

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Gut luminal dysbiosis and pathobiosis result in compositional and biodiversified alterations in the microbial and host co-metabolites. The primary mechanism of bacterial evolution is horizontal gene transfer (HGT), and the acquisition of new traits can be achieved through the exchange of mobile genetic elements (MGEs). Introducing genetically engineered microbes (GEMs) might break the harmonized balance in the intestinal compartment.

Keywords: horizontal gene transfer ; genetically engineered microorganisms ; mobile genetic elements ; regulation ; autoimmune diseases ; microbiome ; dysbiome ; gut ; intestinal

1. Introduction

Many essential functions of the human body depend on the enteric symbiotic microbiota composition and biodiversity, essential components for human health. This intricate host–taxa relationship is a dynamic result of their long-term coevolution. This eubiosis harmonically maintains the host's nutrition, metabolic passways, physiology, protective immune system and even behavior to the extent that we need them and cannot live without them. Greater phyla diversity is associated with microbiota resilience, sustained stability and greater ability to perform metabolic functions. The loss of microbiota phylogenic diversity and enhanced gut dysbiotic composition were associated with the Western lifestyle and several inflammatory, neurodegenerative, neurodevelopmental, infectious, metabolic, cancer and autoimmune diseases (ADs) that put human health at risk [1][2][3][4][5].

The primary mechanism of bacterial evolution is horizontal gene transfer (HGT), and new traits can be acquired through this mobile element exchange. Introducing GEMs might break the harmonized balance in the intestinal compartment [1][6]. The stable temperature, constant physicochemical conditions, continuous food supply, extremely high concentration of prokaryotic cells and phages, and plenty of opportunities for conjugation on the surfaces of host tissues and food particles represent one of the most favorable ecological niches for GEM-originated horizontal gene exchange of detrimental and harmful genetic sequences. Newly developed techniques of bacterial-mediated drug delivery have recently emerged using genetically engineered microbes aiming to locally deliver recombinant therapeutic proteins to the human gut. They are often called live biotherapeutic products, but they deliberately embed potential risks.

2. Numerous Harmful Mobile Genetic Elements (MGEs) Can Be Transferred to the Human Microbiome

Through their genomes, bacteria are subjected to rapid mutations and numerous rearrangements or HGT among and/or within bacterial species. Those MGEs, represented by bacteriophages, transposons, plasmids, and other pathogenic islands, represent a substantial amount of the microbial genome. Applying GEMs to the intestinal lumen can annulate the expression of beneficial genes while inducing the secretion of detrimental proteins. Alternatively, the GEMs can acquire the MGEs in the gut lumen. The following are various major and harmful clinical examples (**Figure 1**, **Table 1**).

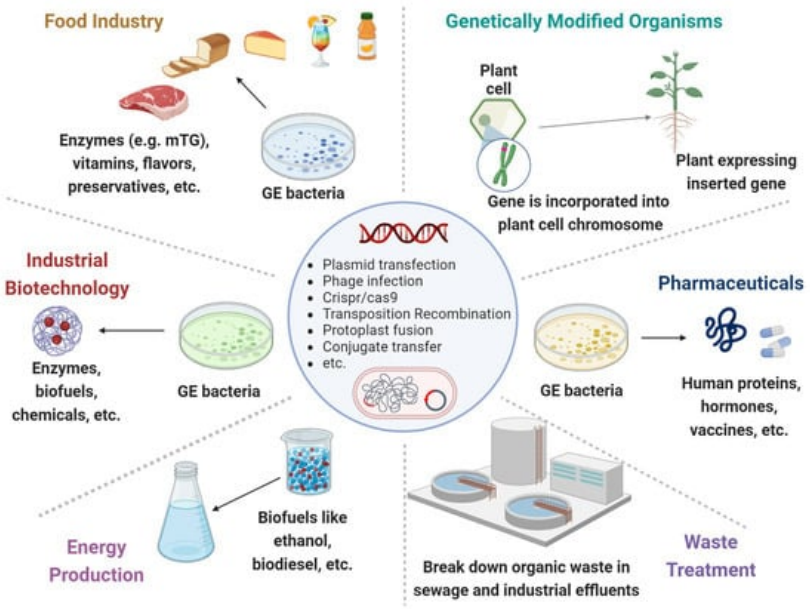

Figure 1. Genetically engineered microorganisms (GEMs) applications. GEMs have a wide range of applications across various fields due to their versatility and the precision of genetic engineering techniques. **Food Industry**: Production of vitamins, flavors, enzymes, and preservatives. They can help in improving the nutritional value, taste, and shelf-life of food products. **Agriculture**: Promote plant growth, increase nutrient uptake, and protect plants from pests and diseases. **Medicine and Health Care:** Cost-effective production of pharmaceuticals, including insulin, growth hormones and vaccines. **Waste Treatment:** Break down hazardous substances like oil spills, heavy metals and other toxic chemicals. **Energy Production:** The production of biofuels like ethanol and biodiesel. **Industrial Biotechnology**: Improve chemical production to increase yields and reduce environmental impacts.

- **Antibiotic resistance genes** (ARGs) and **multidrug resistance (MDR) genes are the most reported** [1][7][8][9]. Less reported but not less important is the development of resistance of bacteria to phages [10][11], drug resistance to cancer therapy [12], resilience against antimicrobial defensive factors [13] and the MDR genes transfer along the food chain, including by contaminated and industrially processed nutrients [14]. The emergence of the resistome represents a worldwide health threat driven by the increasing unnecessary use of antibiotics and anticancer therapy. It occurs mainly by accumulating ARGs and MDR genes on MGEs, which is made possible by HGT [1][15]. Even the frequently consumed *Lactobacillus reuteri* was reported to carry ARGs [16][17]. The ARG does not originate only from human antibiotic consumption—antibiotic residue in food from animal sources can also drive the resistome [14][18]. Most recently, a high rate of ARG carried by Enterobacterales and diarrheagenic Escherichia coli in healthy donors screened for human fecal transplantation was noted [19]. The authors recommended multiplex PCR panels for stool donor screening. One wonders if the GEMs, said to benefit human health, are screened for ARGs or MDR genes.

- **Microbial-engineered enzymes** are an exponentially growing area that has become indispensable to processed food production, pharmaceuticals, and numerous other commercial goods [20]. Despite their beneficial effects on the processed food industries with increased production yields and “enhancing quality and sustainability” [21], multiple scientific publications are calling for a reassessment of their safety [22][23][24][25][26]. Intriguingly, a recent call was to reevaluate the GRAS definition allocated to various processed food additive ingredients. More reliable and updated approaches are offered to enzyme and other food nutritional categories for a more scientifically rigorous, sound and transparent application of the GRAS concept [27][28][29][30][31][32]. Moreover, a call to label, declare utilization and ensure consumer transparency regarding GEM enzymes is expressed in multiple scientific publications [28][33][34][35].

  Many nutritional components and nutrients are treated by GEM enzymes, resulting in post-translational modified proteins, turning naïve peptides into immunogenic complexes [2][30][32][36]. There are multiple examples of genetically engineered microbial enzymes; hence, one example will be expanded, namely the microbial transglutaminase (mTG).

  Microbial transglutaminase is a frequently used processed food additive, and its cross-linked complexes usage is expanding exponentially. The enzyme was classified as a processing aid and was granted the GRAS (generally recognized as safe) definition decades ago, thus avoiding a thorough assessment according to current criteria of toxicity and public health safety [24][25][26][37][38][39].

  In contrast to the manufacturer’s declarations and claims, mTG and/or its transamidated complexes are proinflammatory, immunogenic, allergenic, pathogenic and potentially toxic, hence compromising public health [24][25]

[26]. Being a member of the transglutaminase family and functionally imitating the tissue transglutaminase to demidate or transamidate gliadin peptides, it was recently reported as a potential inducer of celiac disease [26][40][41][42]. In addition, its family member, the tissue transglutaminase, is a well-known inflammation inducer, fibrosis mediator and is heavily involved in sepsis [43][44]. Since mTG functionally imitates its endogenous member, one wonders if it contributes to those morbid conditions.

Microbial transglutaminase and its docked complexes have numerous detrimental effects. Interestingly, in contrast to many publications showing the positive and beneficial aspects of mTG usage [45][46][47][48][49][50], there is evidence for the negative and harmful aspects of enzyme usage that might impact and compromise public health [25][26][28]. The debate between the GRAS category allocated by the FDA regulatory authorities for safe mTG consumption versus many critical scientific publications is ongoing. Several national regulatory committees have warned the public about the hazardous effects of mTGs [24][25][26]. In the case of mTG, it is possible for the gene responsible for its production to be transferred horizontally between microorganisms and even to eukaryotes [1][51][52]. Indeed, MGEs with mTG activity can potentially be transferred by HGT in between prokaryotes. Their presence in a gut luminal cellular compartment presents new opportunities for HGT, with the risk of inhabiting eukaryotic hosts [1][52][53]. One of the hypothetical scenarios is the acquisition of a classic microbial survival factor, such as a Trojan horse, against host self-defense barriers [1][25][26][54]. This gene exchange can happen through mechanisms like plasmid transfer or the incorporation of the transglutaminase gene into a MGE that can be transferred between bacteria. It is worth noting that the specific mechanisms and frequency of HGT for the mTG gene may depend on the particular microorganisms involved and the environmental conditions. The efforts to improve mTG production, thermostability and pH dependency by genetic engineering may do the opposite by enhancing the detrimental effects of the manipulated enzyme [55]. Finally, the fact that mTG is a bacterial survival factor can represent a significant positive selective pressure in the harsh, overcrowded luminal compartment [1][2], enhancing its HGT to other intestinal prokaryotic dwellers. It can be summarized that the mTG acts as a double-edged sword, protecting the microbes to survive in the gut lumen, hence compromising human health [24][25][26][54].

- The place of **probiotic** consumption should be highlighted in terms of their side effects. Drug resistance remains a universal threat, and the fad of probiotic consumption, many of which contain antibiotic-resistant elements, is a major and serious health concern [56][57][58][59]. In 2023, emerging issues in probiotic safety arose. Whole-genome sequencing should be implemented to detect virulence factors, toxins, ARGs and other detrimental MGEs [60]. The clear assignment of species and strain identity risks to vulnerable populations and the need for adverse event reporting are important topics to regulate.

  Engineered probiotics through gene editing is an emerging domain. Despite the reported clinical benefits for inflammatory bowel disease, infectious, tumor and metabolic diseases, tight regulatory measures are lacking [61]. Engineered and naïve probiotics compete with the luminal microbiome for nutrients or ecological niches and thus might affect the diversity and composition of intestinal microbiota. Human health can be more affected by their interaction with the luminal lipid metabolism [62]. Once again, consumer transparency, visible labeling and safety regulations are far from satisfactory.

- **Genetically modified (GM) plants** might possess beneficial traits like resistance to drought, pests and diseases, fighting climate change, improved agricultural and industrial production and enhanced nutrition. However, it also has a risky side to humans, animals and environmental health that should be regulated by national food security and regulatory authorities [63]. Mobile elements such as modified DNA can be laterally transferred to other recipients, spanning prokaryotes, eukaryotes and even to people [1][63]. More so, delaying tightened regulation risks facing increased GM plants, including genome-edited crops with deliberately altered and potentially harmful sequences [64][65][66]. A call for reconsideration before consumption [67], problematic and insufficient national legislation [68], risk of allergenicity [69] and consumer's knowledge versus fears [70][71] are increasingly expressed concerning genetically modified food. **Table 1** summarizes the harmful MGEs that potentially can compromise public health.

Table 1. MGEs harmful effect that can compromise public health.

MGEs	Potential Harmful Effects	References
Antibiotic resistance or multidrug resistance genes	**Microbial antibiotic resistance Bacterial resistance to phages Drug resistance to cancer therapy Resilience against antimicrobial defensive factors Contaminated and industrially processed nutrients Potential entry to the human genome by HGT**	**[1][2][3][4][5][6][7][8][9][10][11][12][13][14]**

MGEs	Potential Harmful Effects	References
Microbial-engineered enzymic genes with MTG as an example.	Post-translational modified proteins, turning naïve peptides into immunogenic complexes Complexes are proinflammatory, allergenic, pathogenic and potentially toxic, hence compromising public health Potential inducer of celiac disease	[2][11][24][25][26][28][33][34][36][41][42][72][73][74][75][76][77]
	MGE presence in a gut lumen presents new opportunities for HGT, with the risk of inhabiting eukaryotic hosts	[1][52][53]
	Transfer of microbial survival factors against host self-defense barriers	[1][25][26][54]
	Improved enzyme production, thermostability and pH dependency by genetic engineering might enhance the detrimental effects of the manipulated enzyme	[55]
Probiotics containing MGEs	Transfer of drug resistance Transfer of virulence factors, toxins, ARGs and other detrimental MGEs should be implemented	[56][57][58][59][60][61]
	Interference with the luminal lipid metabolism	[62]
	Modified DNA or other MGEs can be laterally transferred to other recipients, spanning prokaryotes, eukaryotes and even people.	[1][63]
Genetically modified plants	Genome-edited plants, like crops with deliberately altered and potentially harmful sequences, can invade the human microbiome or genome	[64][65][66][67][68][69][70][71]

GEMs—genetically engineered microorganisms, HGT—horizontal gene transfer, MGEs—mobile genetic elements, mTG—microbial transglutaminase, ARGs—antibiotic resistance genes.

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