FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 10 No. 10, May, 2026, pp 276 – 287
DOI: https://doi.org/10.33003/fjs-2026-1010-5236

Applications of Genetically Engineered Microorganisms in Medicine, Food, Environment and Agriculture

***[1]Gideon Yakusak Benjamin and [2]Rejoice Ziriyi Benjamin**

[1]Department of Applied Biological Sciences (Microbiology Unit), State University of Medical and Applied Sciences, Igbo-Eno, Enugu State, Nigeria
[2]Department of Biological Sciences, Kaduna State University, Kaduna State, Nigeria.

*Corresponding authors' email: gideonbenjamin.y@gmail.com

ABSTRACT
Genetic engineering is the artificial manipulation and recombination of DNA or other nucleic acid molecules in order to alter the genetic makeup of an organism, typically to introduce or modify specific traits. Different types of genetically engineered microorganisms (GEMs) have been developed through recombinant DNA and RNA technologies; these have been applied in the field of medicine, food industry, environment and agriculture. GEMs have been utilized in medicine for the production of antibiotics, vaccines and heterologous proteins such as insulin. Microorganisms are used in food production either as integral parts in the preparation of various fermented foodstuffs or to produce food additives and processing aids (organic acids, flavouring agents, food enzymes). Modifications are made to the genetic makeup of microorganisms to either produce a new protein or other food ingredient, to improve or enhance the production of an existing protein/ingredient, or to tailor the characteristics of an existing protein to a new application. Genetically engineered microorganisms can be used for the bioremediation of polluted soils and therefore increase soil productivity. They can also be used as bio-detectors to monitor soil pollution so that it can be managed more efficiently or prevented. They can also enhance nitrogen fixation and nutrient uptake in the soil. Microbial biological control agents (MBCAs) are an example of GEMs that could be used in agricultural practices to make them more environmentally friendly and less polluting. The present study reviews the applications of genetically engineered microorganisms in medicine, environment, food and agriculture.

Keywords: Genetic, Transgenic, Microorganisms, Agriculture, Environment

INTRODUCTION

Genetic engineering is the use of molecular biology technology to modify DNA or other nucleic acid molecules in order to alter the genetic makeup of an organism, typically to introduce or modify specific traits (Lanigan *et al*.,2020). It involves the use of laboratory tools to change the nucleic acid sequence of an organism by removing or adding base pairs, inserting or inactivating an unnecessary virulence gene; creating a new characteristic in microorganisms. Genetically modified organisms (GMOs) are plants, animals, or microbes that have had their DNA changed using genetic engineering techniques. Genetically Engineered Microorganisms (GEMs) also known as Genetically Modified Microorganisms (GMMs) are microorganisms (bacteria, molds and yeasts) whose genomes have been engineered or modified in the laboratory in order to favour the expression of desired physiological traits or the generation of desired biological products. Hence, micro-organisms can be genetically altered to add or enhance certain properties, giving them a wider range of application than regular microbial products. The *Bacillus thuringiensis* bacterium, for instance, can be modified to produce an additional toxin that originates from a related strain. This allows the bacterium to be used as a pesticide against not only harmful caterpillars, but also harmful species of fly. Organisms may also be modified to retain their effectiveness under unfavourable weather conditions. So far, only a few genetically modified micro-organisms are commercially available outside Europe as plant protection products (Scheepmaker*et al*., 2016). Insulin is now produced in microbes rather than sacrificing pigs to harvest their pancreas, the original source of insulin. Many other techniques such as chemical mutagenesis and interspecies crossing can be used to modify the genetic makeup of a microorganisms, but not all of these techniques fall under regulatory definitions of genetically engineered or genetically modified (NRC, 2004).

In conventional livestock production, crop farming, and even pet breeding, it has long been the practice to breed selected individuals of a species in order to produce offspring that have desirable traits. In genetic modification, however, recombinant genetic technologies are employed to produce organisms whose genomes have been precisely altered at the molecular level, usually by the inclusion of genes from unrelated species of organisms that code for traits that would not be obtained easily through conventional selective breeding (Fridovich-Keil and Diaz, 2024).

With recent advances in genome sequencing and genetic techniques, it is possible to make alterations in the gene sequence of phages and a wide range of bacterial strains to introduce new strains applied for the prevention, diagnosis or treatment of an infection (Waksman, 2019).Various mutations, transformation, conjugation, protoplast fusion, electroporation, recombination technology, and molecular genetics are commonly used in genetic engineering. In the past and before molecular genetics methods were invented, researchers used mutations induced by UV radiation and chemicals (Hashimoto *et al*., 2015). Through a combination of biotechnology and genetic engineering science, there are three gene-editing tools including zinc finger nucleases (ZFNs) technology, transcription activator-like effector nucleases (TALEN) technology, and clustered regularly interspaced short palindromic repeats-CRISPR associated (CRISPR-Cas) technology as first, second, and third generation technologies in recent years for gene editing by double-strand breaks in desired sequence of genes. The ZFNs and the TALEN are restriction endonuclease-based systems that show limitations such as inducing nonspecific mutations and are time consuming, expensive, and nonspecific methods

(Li *et al*., 2017), while the CRISPR technology is a more efficient and powerful editing method with more flexibility and simplicity (Citovsky *et al*.,2007).

Techniques for Gems Production

There are different methods to modify microorganisms for their application in medical and life sciences. In microbial genetic engineering, target genes are first sheared, spliced, and integrated using genetic operation tools before being inserted into chassis cells. Recombinant genes are therefore incorporated into the intended products or provide the bacterium with new phenotypes (Ari *et al*., 2024). Techniques used for the genetic manipulation of bacteria include CRISPR-Cas9, molecular cloning and recombinant DNA technology, bacterial artificial chromosomes (BACs), transformation, transduction, phasmid transfection, protoplast fusion, conjugation, and transposition recombination, Transduction, Gene knock-out/knock-in, Polymerase Chain Reaction, Blue white screening, Homologous recombination (Ramesh *et al*., 2006).

Applications of GMMs in Medicine and Pharmaceutical Industries

Many microorganisms (bacteria, viruses, and fungi) have a high potential to treat diseases such as HIV infection, colitis, and cancer trough applying genetic changes to them. Engineered viruses can be used in treatment of cancer and hereditary vision loss due to retinal dystrophy and in prevention of alcoholism (Nemunaitis *et al*., 2006; Wiese *et al*., 2009). Various applications of engineered bacteria in different fields include combating specific pathogens, stopping the emergence of antibiotic resistance, electrical conductivity, and maintenance of uric acid levels in treatment of obesity, as well as removal of pollutants in nature. Prevention of malaria transmission through restraining sporozoites from sticking to salivary glands is one of the applications of bioengineered fungi in the medical field (Ghosh *et al*., 2009).

Antibiotics Production

The widespread use of antibiotics in medicine, veterinary, and agriculture causes the emergence and spread of antibiotic-resistant bacteria. With the increase in bacterial infections caused by pathogens that are resistant to one or more antibiotics, the world has entered another challenge, these challenging pathogens include *E. coli*, *S. aureus*, *P. aeruginosa*, *Acinetobacter baumannii* (*A. baumannii*), *Klebsiella pneumoniae (K. pneumonia*), and *E. faecium* (Egeland *et al*., 2011). In addition to being resistant to antibiotics, these mentioned bacteria are able to spread this resistance to other antibiotic-sensitive strains. This is a worrying issue and should be replaced by newer treatment methods. One of these new methods is genetic engineering and synthetic biological sciences, which with the conceptualization and protein engineering and in silico design will ultimately lead to the development of new treatment methods that combat antibiotic resistance. genetic engineering technology with recombinant DNA tools are widely used to genetically manipulate antibiotic-producing microorganisms to produce more antimicrobial compounds (Gorlach, 1994).

Mutation and genetic engineering technology with recombinant DNA tools are widely used to genetically manipulate antibiotic-producing microorganisms to produce more antimicrobial compounds. The main goal of genetic engineering in antibiotic-producing microorganisms is the synthesis of new strains of microorganisms that produce the desired antibiotic in larger quantities by changing antibiotic biosynthesize pathways to improve the rate of antibiotic production and synthesis of hybrid and new modified antibiotics (Gorlach, 1994, Mahdizade, *et al*., 2024).

Vaccinology

Vaccines are the main strategy for preventing most infectious diseases. Nonpathogenic bacteria can be changed by genetic engineering to express antigens on their surface, resulting in stimulation of the immune system. Such GEMs provide long-lasting immunity and stable protection in constructed vaccines such as recombinant vaccinia virus and herpesvirus of Turkey (HVT) modified to prevent rabies and Marek's diseases by vaccination (BOC, 2026).

Advancements in vaccine technology are increasingly focused on leveraging the unique properties of both pathogenic and commensal bacteria. This revolutionary approach harnesses the diverse immune modulatory mechanisms and bacterial biology inherent in different bacterial species enhancing vaccine efficacy and safety. Pathogenic bacteria, known for their ability to induce robust immune responses, are being studied for their potential to be engineered into safe, attenuated vectors that can target specific diseases with high precision (Lloren *et al.,* 2024).Since commensal bacteria colonize the host without causing disease, recombinant commensal bacteria can be engineered to express antigens from pathogens. For example, *Lactobacillus casei* has been engineered to express antigens from Rotavirus which induced protective immunity when administered orally (Zhang *et al.,* 2022a). *Lactobacillus* strains are engineered to express tetanus toxin fragment C (TTFC) which when administered via mucosal route, will induce antigen-specific IgG and IgA antibodies against tetanus (Grangette *et al.,* 2001). *Bifidobacterium longum* has been used to deliver *Clostridium difficile* toxin B fragment against *C. difficile* infection (Wei *et al*., 2018).

In recent years, major strides have been made in exploiting bacterial vectors to deliver plasmids or antigens into the host cells (Schoen *et al*. 2004). Some examples of well-studied attenuated bacteria serving as carriers for DNA vaccines include *Salmonella* spp. (Liang *et al.,* 2014), *Shigella* spp (Xu *et al.,* 2003), *Yersinia enterocolitica* (Al-Mariri *et al.,* 2002), and *Listeria monocytogenes.* Additionally, non-pathogenic lactic acid bacteria such as *Lactococcus* spp., *Streptococcus* spp., and *Lactobacillus* spp. have also been explored as promising candidates in DNA vaccine delivery systems. These bacterial vectors offer versatile platforms for DNA vaccine delivery, with the potential to elicit robust immune responses against targeted pathogens or antigens. Careful consideration in selecting bacterial strains is essential, as each bacterial species possesses unique immune modulatory mechanisms and intracellular lifestyles such as invasion, replication and dissemination. For example, *Salmonella* features an intravacuolar lifestyle (Röder *et al.,* 2021) whereas *Listeria* demonstrates an intracytoplasmic lifestyle

Genetically engineered vaccines are applied in the prevention of infectious diseases, therapeutic cancer vaccines, including Sipuleucel-T (Provenge), have been genetically modified to activate the immune system to identify and eliminate cancer cells, the vaccines are frequently tailored according to certain tumor markers or antigens. Examples of genetically engineered vaccines include; the genetically engineered vaccine for infectious hematopoietic necrosis virus (IHNV), this vaccine offers substantial protection to rainbow trout against both waterborne and injectable challenges in fish weighing between 2 and 160 g. Vaccine effectiveness trials in Atlantic salmon and other commercially relevant species also

shown markedly high levels of protection against IHNV. Another example is the AstraZeneca (ChAdOx1-S) COVID-19 vaccine. It is a viral vector vaccine utilizing a genetically engineered chimpanzee adenovirus to express the SARS-CoV-2 spike protein. This vaccine, similar to others in its category, utilizes a non-replicating viral vector to effectively introduce the antigen into host cells, therefore eliciting a protective immune response. The Hepatitis B Vaccine is among the first genetically designed vaccines, utilizing recombinant DNA technology to generate the hepatitis B surface antigen (HBsAg) in yeast cells, therefore eliciting protective immunity against hepatitis B. Potential for immune escape and genomic integration are the few limitations of genetically engineered vaccines.

Production of Heterologous Proteins

Heterologous expression is the introduction of either complementary DNA (cDNA) or RNA (cRNA) encoding for a protein of interest from one species into the cell of another species, such that the hosts' cellular machinery expresses the foreign protein (Gagnon, 2009). In 1982, insulin, expressed from human insulin genes on plasmids inserted into *Escherichia coli*, was the first genetically engineered therapeutic agent to be approved for clinical use in humans. Human growth hormone (hGH), a protein made naturally by the pituitary gland, was the second of such product to be approved for clinical use in humans. Inadequate secretion of hGH in children results in dwarfism. Before the advent of recombinant DNA technology, hGH was prepared from pituitaries removed from human cadavers (Glazer and Nikaido, 2007).

Engineered Microorganisms for the Treatment of Cancer and Inflammatory Diseases

As the science of genetic engineering advances, researchers have turned their attention to target tumors using genetically engineered bacteria. There are several types of anaerobic bacteria such as *Salmonella typhimurium, Clostridium, E. coli*, and *Bifidobacterium* known as live biotherapeutic products (LBPs) that are able to survive in the tumor site due to their anaerobic nature and adaptation to hypoxic condition. They would have many benefits for cancer treatment by their ability to spread and carry the drug, anticancer compounds, proteins, and pre-enzymes to the anaerobic space of the cancer mass (Pan *et al*., 2021). They have many potential benefits for cancer treatment related to their ability to spread and to transfer and release drug or anticancer compounds to anaerobic space of the cancer mass (Ghanavati *et al*., 2020; Pan *et al*., 2021). Following genetic modification of the bacteria to harbor genes and proteins, colonization in tumor site occurred, resulting in not only transfer of antigens or cancer-fighting compounds but also stimulation of the immune response (Parisa *et al*., 2020).

GEM is used as a new method in the treatment of many diseases especially inflammatory diseases (Alvarez and Fernandez, 2017). Engineered *L. lactis* strains were designed by Steidler *et al*. (1979) to produce IL-10 as an anti-inflammatory cytokine. Results of their study showed 50% reduction in colitis symptoms in murine chronic colitis model induced by dextran sulfate sodium (DSS). In addition to being cost-effective, this treatment method also causes localized delivery and an active synthesis in situ. Engineered *Bifidobacterium* spp. (*B. longum*), also have been developed for the treatment of inflammatory bowel disease (IBD) to deliver α-melanocyte-stimulating hormone (α-MSH) exhibiting an anti-inflammatory effect on the intestine. Well intestinal colonization with engineered *B. longum* and significant anti-inflammatory effects by high α-MSH expression were observed in rat model of ulcerative colitis. Actually, this engineered bacterium reduces IL-6, TNF-α, nitric oxide (NO), and myeloperoxidase enzyme from which all are proinflammatory factors in ulcerative colitis and also increases IL-10 as an anti-inflammatory cytokine (Dosoky *et al*., 2020).

Another strategy used in the treatment of colitis is the expression of enzymes with antioxidant properties. For instance, *Streptococcus thermophilus* (*S. thermophilus*) strains genetically modified by a plasmid to express catalase and superoxide dismutase were able to reduce colitis in a mouse model by reducing reactive oxygen species (ROS) production. So, these findings suggest that genetically altering a bacterium candidate with inherent immunomodulatory capabilities (*S. thermophilus* CRL 807) by inserting a gene encoding an antioxidant enzyme improves its anti-inflammatory effects to express catalase and superoxide dismutase and is able to reduce colitis in a murine model

In addition to bacteria, bacteriophages can also be used in genetic engineering technology to express anti-inflammatory peptides on the surface of phages for the treatment of inflammatory diseases (Zhang *et al*., 2022b). Several anti-inflammatory medications have been created over the years to lower the concentrations of inflammatory mediators in the body, for the treatment of inflammatory illnesses such as rheumatoid arthritis and carditis, as well as cancer (Zhang *et al*., 2022b).

One of the applications of modified bacteria is in treatment of obesity. This chronic metabolic disorder considerably increases the risk of cardiovascular diseases and diabetes. In addition, since obesity is associated with inflammation, it may lead to autoimmune diseases such as IBD. Surgery and drugs commonly are used for treatment of obesity, but due to the long-term impacts of the current diets and lifestyles, these treatments are not responsive. Transformation of *E. coli* by the N-acyltransferase gene obtained from *Arabidopsis thaliana* (*A. thaliana*) resulted in development of a new strain called At1g78690 to prevent the absorption of fats through different mechanisms such as oxidation of fatty acids and reduction of food consumption by expressing N-acylphosphatidylethanolamines (NAPEs) as precursors of N-acylethanolamide (NAE), leading to obesity control (Chen *et al*., 2014).

Another important disorder is uremia, which occurs following increase in uric acid levels resulting from the decrease in the excretion rate and subsequent accumulation in the kidney (Montefiori, 2015). Uremia mostly is caused by renal failure, diabetes, and excessive consumption of alcohol. One approach for maintaining normal uric acid levels is oral administration of genetically engineered *E. coli* DH5 cells (Rodríguez *et al*., 2001). The urease gene isolated from *Klebsiella aerogenes* (*K. aerogenes*) was inserted to the *E. coli* DH5 strain. The *E. coli* strain was genetically modified to form a sodium alginate-encapsulated semipermeable membrane to persist throughout the gastrointestinal tract. Then, urea molecules quickly spread inside the microcapsules containing bacteria, resulting in reduction of urea amount. These modified bacteria orally were administered and completely excreted through the feces, indicating the safe use of this strain (Piñero-Lambea *et al.*, 2015)

Various species of genetically engineered bacteria including *E. coli* species and some probiotics showed promising results in reducing hyperglycemia and improving diabetes. For example, genetically engineered *E. coli* species can produce insulinotropic proteins including GLP-1 or

pancreatic *β*-cells-specific transcription factor PDX-1 from pancreatic *β*-cells and intestinal cells. Similar studies used an engineered *Lactobacillus* to reduce hyperglycemia in mice through secreting GLP-1. Also, some bacteria especially *E. coli* Nissle strain increased glucose uptake by pancreatic endocrine cells with upregulation of Notch associated with the ngn3 gene by using expression GLP-1 or PDX-1(Duan *et al*., 2015).

Applications of GMMS in Food Production

Microorganisms have been used throughout human history to aid in the production of food, even before humans knew that these organisms were responsible for the fermentation processes involved (Zhang *et al*., 2017). Microorganisms are an important part of the food industry as these are helpful in food preservation and production. Usually, microorganisms are used in making dairy products (yogurt and cheese), fermented vegetables (olives, pickles, and sauerkraut), fermented meats (salami), and sourdough bread. These are also utilized for the production of wine and several other beverages. Recently in the food industry, the use of microorganisms has started on a large scale for the production of chocolate, food color, from preserving fruits, vegetables and meat, and as probiotics which are helpful for human health. Different types of the microorganisms produce enzymes of nutritional value such as microbial transglutaminase for fish production. As the human population is increasing, we need to adopt new techniques for producing qualitative and nutritious food. These microorganisms can be used to cope with the shortage of food supply (Mazhar *et al*., 2022).

Microorganisms (bacteria, yeasts and filamentous fungi) are used in food production either as integral parts in the preparation of various fermented foodstuffs or to produce food additives and processing aids (organic acids, flavouring agents, food enzymes) (Wright and Bruceb, 2003). Modifications are made to the genetic makeup of microorganisms to either produce a new protein or other food ingredient, to improve/enhance the production of an existing protein/ingredient, or to tailor the characteristics of an existing protein to a new application (Hanlon & Sewalt, 2020).

Microorganisms, typically through production of their endogenous enzymes, have been critical to food production for thousands of years for foods like beer, yoghurt, kefir, cheese, soy sauce, wine, vinegar, and many more. Following the discovery of the microorganisms responsible in the 1830s (Barnett, 2003), food producers began examining ways of harnessing these microorganisms to make them more efficient. While it took thousands of years for humans to discover the microorganisms responsible for the existence of many foods, once discovered it only took approximately one hundred years (and the discovery of DNA) for humans to begin using techniques to optimize the use of microorganisms, including the modification of their genetic makeup to make food production using microorganisms more efficient.

In comparison with traditional agricultural production of plant-based substances, such as stevia extracts and vanilla, GEM-based production of steviol glycosides (Philippe *et al*.,2014) and vanillin (Brochado *et al*., 2010) has many sustainability benefits including a reduction in land usage, production of less waste, and provision of a more stable and affordable supply to meet the rising consumer demand.

Role of Lactic Acid Bacteria

Lactic acid bacteria (LAB) are Gram-positive bacteria that produce lactic acid as the main end-product of glucose fermentation. LAB are classified according to their phenotypic and metabolic characteristics such as cellular morphology, growth conditions (as temperature and sugar utilization) and the production of substances to inhibit the proliferation of other microorganisms during the fermentation process. LAB are members of the phylum Firmicutes, and order Lactobacillales (Mokoena, 2017), they are comprised of the genera *Lactobacillus, Lactococcus, Streptococcus, Enterococcus, Leuconostoc, Carnobacterium, Oenococcus, Pediococcus, Tetragenococcus, Vagococcus,* and *Weissella*. Due to their historical long-term use they are regarded as safe, and some of the species are used as probiotics, as they have beneficial effects on people. Moreover, when describing their characteristics, LAB are often claimed to have a 'generally recognized as safe' (GRAS) status, which confirms their safety.

Lactic acid bacteria are being used in a number of food production and storage methods in the modern food industries. Lactobacilli are commonly used for the storage of uncooked fermented sausages, hams and sliced meats to avoid pathogenic *Listeria* infection (McIntyre, 2012). These bacteria have replaced the chemical additives such as sodium lactate and potassium acetate which were used for the safety and quality of the above mentioned meat products; however, these additives leave numb mouth feel or warmed over flavor upon eating (Vermeiren, 2006).

The basic mechanism of action of lactic acid bacteria in raw fermented sausages is the conversion of sugars to lactic acid through fermentation. This contributes to the unfavorable conditions for the growth of pathogenic and spoilage microorganisms. Other than the production of lactic acid, these bacteria produce small amounts by products such as acetic acid, acetoin, ethanol and pyruvic acid which act as the precursors for production of other compounds besides adding aroma to the food (Fontana *et al*., 2012).

Genetic engineering of LAB offers various tools to improve the strains and to enable greater viability and stability, and production and growth rates. The possibilities to effectively express therapeutic proteins and to use LAB as vaccines further strengthens their potential uses (Chowdhury *et al*., 2014). However, no matter how effective a specific GM-LAB might be, a drawback that stands in the way of its marketing is that it has been genetically manipulated; this is mainly due to low consumer acceptance of GM microorganisms (particularly in the European Union) and regulatory restrictions as to their use. At the combined 2018 International Probiotics Association World Congress and Probiota, it was noted that GM probiotics would be perfect from a scientific point of view, and would probably not encounter problems with their registration with the European Food Safety Authority (EFSA).

GMM Used for Optimization of Food Enzyme Production

The use of microorganisms for food enzyme production has the following advantages: (I) Many microbes produce enzymes extracellularly, which simplifies the extraction method during the purification. Even when the production of the enzyme is intracellular, the disruption of microbial cells is easier than the extraction from plants and animals (Robinson, 2015). (II) Particular microbial strains can be selected to obtain well-characterized enzymes with specific properties (Chang *et al*., 2016). (III) Due to a shorter production time, smaller production facilities are needed, resulting in a reduced production cost. Additionally, animals and plants need to be

transported to extraction facilities, which are not needed for MB fermentations, where the whole production takes place in only one location (Hjort, 2007). (IV) Higher yields can be obtained for MB enzymes. Moreover, the availability of enzymes from animal and plant sources is also depended on the time of the year, therefore reducing the total yield and limiting the ability for continuous production (Singh *et al*., 2016). (V) Microbial enzymes usually have a higher activity and stability. For instance, enzymes obtained from thermophilic microorganism will have a higher temperature tolerance (Vieille and Zeikus, 2001). (VI) Microorganisms can easily be genetically modified in order to obtain optimized enzyme products, higher yields, and better characteristics. Additionally, modifications of animals and plants are technically more difficult, and modifications of animals, especially, are ethically more sensitive (Liu and Kokare, 2017)

Considering the different advantages of using microorganisms for food enzyme production, the ease of introducing genetic modifications in the production microbial strains to further improve the industrial enzyme production is probably one of the biggest advantages. These modifications can be obtained by either the genetic modification of the production strain or by the use of recombinant enzymes. The use of genetically modified microorganisms for the production of food enzymes allows the production yield to be increased, improving the characteristics of the produced enzyme, limiting the production of unwanted metabolites (such as mycotoxins), and expressing enzymes in organisms that normally would not produce this enzyme (Olempska-Beer *et al.*, 2006). Recombinant enzymes are used to improve an enzyme's characteristics, such as its activity, temperature optimum, and pH stability (Trono, 2019).

Nisin production by genetically modified Lactococcus lactis

The antimicrobial peptide nisin is composed of 34 amino acids that are produced during the growth of various *L. lactis* strains. Generally, nisin inhibits and prevents the spread of Gram-positive bacteria. This constitutes an important element to be used as an excellent food preservative and additive in the food industry (Chen *et al*., 2023).

The analysis of nisin biosynthesis shows the way for carrying out genetic modifications designed to raise nisin yield. The genetic modifications were centralized on the issues that limit the production of nisin. Innovative genetic approaches have increased nisin production with *L. lactis* strains expressing the *aox1*, *pfk13*, and *pkaC* genes (Papagianni *et al*., 2012). These recombinant nisin producers induced oxidative respiration and glycolytic activity. Additionally, nisin Z production was improved through the use of protoplast fusion and genome shuffle in *L. lactis* ssp. *lactis* YF11. By simultaneously expressing the *hdeAB*, *Idh*, and *murF* genes, the nisin production of the producer *L. lactis* was improved, as was its acidic tolerance. Herewith, over-expression of the *asnH* gene increased the acidic tolerance in the nisin producer *L. lactis* (Portieles *et al*., 2023). Thus, genetic engineering of *L. lactis* has shown to be very helpful in increasing both yield and effectiveness of nisin.

Genetically Modified Probiotics

Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host (Binda *et al*., 2020). Probiotics play a great role in many aspects, such as preventing and treating various clinical diseases, improving the intestinal microenvironment, inducing immune regulation, preventing physiological stress, inhibiting the growth of pathogens, and improving the barrier function of the intestinal epithelium, establish homeostasis in the microbial flora, modulate the immune response and metabolic pathwaysetc (Suez *et al*., 2019; Sedighi *et al*., 2019). Considering that the impacts of probiotics are significantly related to the type of species and its prescribed dose, their performance can be attributed to their ability to inhibit the colonization of pathogens, establish homeostasis in the microbial flora, and modulate the immune response and metabolic pathways (Sedighi *et al*., 2019). Genetic engineering can be helpful in the reduction of probiotic pathogenicity and reinforcement of their useful properties. In addition, genetic engineering can make probiotics safe for human applications such as vaccination and delivery of target proteins and drugs (Sedighi *et al*., 2019). Bioengineered probiotics would be more useful in the diagnosis and treatment of specific diseases

An engineered *Lactobacillus casei* strain that can produce *Listeria* adhesion protein was developed by an American team in the year 2020. This strain is colonized in the intestine of mice, competitively reducing the colonization of *Listeria* mucosa and systemic transmission, protecting mice from fatal infections. They can also enhance the intestinal immune regulation function by accumulating intestinal mucosal regulatory T cells, $CD11c^{+}$ dendritic cells and natural killer cells. The method of engineering *L. casei* ATCC334 to reduce *Listeria* infection and protect the intestinal tract is worth learning. Some researchers have designed and constructed an engineered probiotic based on *E. coli* Nissle 1917, which can specifically target and kill the two most common vancomycin-resistant *Enterococcus*, and significantly reduce the number of *Enterococcus faecalis* and *Enterococcus faecium* in the feces of model mice (Geldart *et al*., 2018). Some researchers have engineered *Saccharomyces boulardii* so that the engineered bacteria secrete a fusion protein (ABAB) that can neutralize 4 different *Clostridium difficile* toxins (Chen *et al*., 2020). The preventive administration of these bacteria can significantly relieve the inflammation and tissue damage related to the infection of *Clostridium difficile* in the mouse intestines mucosa, therefore reduce the mortality of these mice. In addition, researchers have developed some engineered probiotics to better prevent and prevent and diagnose bacterial infections. Some researchers have genetically modified *Lactobacillus lactis* to produce engineered probiotics that recognize the cholera autoinducer 1 (CAI-1) produced by *Vibrio cholerae* and cause color changes in feces (Mao *et al*., 2018). Another researcher has constructed an engineered *lactic acid bacteria* that can be used to detect real-time changes in autoinducer peptide-I (AIP-I) produced by *Staphylococcus aureus* (Lubkowicz *et al*., 2018). The researchers also engineered *E. coli* Nissle 1917 to specifically kill pathogens by detecting the autoinducer N-acyl homoserine lactone (AHL) of *Pseudomonas aeruginosa* and releasing antibiotics and anti-biofilm enzymes (Hwang *et al*., 2017). This series of studies may provide a new direction for the treatment and prevention of drug-resistant bacterial infections. It is hoped that the safety and effectiveness of these engineered probiotics can be further confirmed, and these products can serve the clinic as soon as possible.

Applications of Genetically Modified Microorganisms in Environment

It is known that microorganisms play a vital role in remediation of soil pollutants like heavy metals, pesticides, hydrocarbons, etc. However, the indigenous microbes have a limited capacity to degrade these pollutants and it will be a slow process. Genetically modified microorganisms can

catalyze the degradation process as their altered metabolic pathways lead to hypersecretions of various biomolecules that favor the bioremediation process (Rebello *et al.*, 2021).

Researchers try to insert specific novel genes that do not exist in nature and have high degradation capacity as compared to wild microbes. GMMs are more powerful in their degradative potential than wild microbes because they can easily acclimatize themselves against new pollutants. Hence, GMMs can give an alternative solution to degrade complex waste like toluenes, oil spills, halobenzoates, naphthalenes, trichloroethylene, xylenes, and octanes etc. instead of wild strains that degrade complex compounds very slowly (Rafeeq *et al.*, 2023). The use of GMMs for waste management and sustainable agriculture offer benefits in both eco-friendly and cost-effective ways as compared to available conventional technologies. GMMs reduces the need for additional fertilizers, pesticides, and herbicides which promote plant health and can increase agricultural production and soil productivity. GMMs can enhance nitrogen fixation, and nutrient uptake from the soil and could be used for the control of diseases, weeds, or pests in crop plants to make them more environmentally friendly.

GMMs are used in the management of environmental issues. For example, some bacteria can produce biodegradable plastics, and the transfer of that ability to microbes that can be easily grown in the laboratory may enable the wide-scale "greening" of the plastics industry. In the early 1990s, Zeneca, a British company, developed a microbially produced biodegradable plastic called Biopol (polyhydroxyalkanoate, or PHA). The plastic was made with the use of a GM bacterium, *Ralstoniaeutropha*, to convert glucose and a variety of organic acids into a flexible polymer. GMOs endowed with the bacterially encoded ability to metabolize oil and heavy metals may provide efficient bioremediation strategies (Fridovich-Keil and Diaz, 2024).

Table 1: List of Recombinant Microbes with Different Xenobiotic Compounds

Name of the microbe	Type of xenobiotic removed	Significant features
Caulobacter crescentus JS4022/p723-6 H	Heavy metals like cadmium	Over expresses hexahistidine peptide on the surface of the bacterial cells. Acid treatments help to recover metals as the microbe is acid tolerannt
Escherichia coli DH5α	Uranium and chromium	Hydrogels containing engineered metalloproteins, super uranyl-binding proteins (SUP), and naturally occurring molybdate/chromate binding proteins (ModA)
Apostichopus japonicus (AjFER)	Cd^{2+}, Hg^{2+}, Cr^{3+}, Pb^{2+}, and As^{3+}	Recombinant ferritin
Escherichia coli	Cadmium	*Neurospora crassa* metallothionein protein
Escherichia coli	Cd, As, Hg, and Zn	Recombinant sheep metallothionenin protein fused with the maltose binding protein (MBP)
Recombinant *Rhodococcus erythropolis*	Removes nitrogen and organic matter in landfill leachate	Hydroxylamine oxidase (HAO) and ammonia monooxygenase (AMO) genes (rRH-HA)
Recombinant *Deinococcus radiodurans*	Cadmium and uranium bioaccumulated in cytoplasm	*Synechococcus elongates* metallothionein protein expressed in S Layer proteins Hpi and SlpA of Deinococcus
Indigenous bacteria of soil	Hydrocarbon degradation	Catabolic genes for petroleum hydrocarbon degradation from *E. coli* transferred by mating
Acinetobacter baumannii S30 pJES	Hydrocarbon degradation	Degradation by lux-tagged *A. baumannii* S30 pJES
Streptomyces coelicolor M145	n-Hexadecane degradation	Over-expressing *alkB* gene encoding for the enzyme alkane monooxygenase
Acinetobacter sp. BS3	Aromatic hydrocarbons	Insertion of xylE gene encoding for catechol 2,3-dioxygenase enzyme from *Pseudomonas putida* strain BNF1
Protoplast fusion *of Sphingomonas* sp. *GY2B and Pseudomonas* sp. *GP3A*	*High capacity of degrading phenanthrene*	Random fusion done
Escherichia coli	Atrazine pesticide degradation	Hydrolase producing gene-based recombinant
Sphingomonas sp. BHC-A	Hexachlorocyclohexane (HCH) and methyl parathion degradation	Methyl parathion hydrolase gene (mpd)
Pseudoalteromonashaloplanktis TAC125	Wide range of aromatics	Toluene-o-xylene monooxygenase coding gene

Source: (Rebello *et al*., 2021)

Applications of Genetically Modified Microorganisms in Agriculture

In agriculture, the goal of genetic modification is to provide enhanced features to plants by altering their genetic makeup. This is done by inserting a gene into the genome of a plant. FlavrSavr tomatoes were the first genetically modified plants, and they were modified to delay the ripening process, preventing tenderness and rot. Complete crop production increased significantly after the introduction of GM crops at some point during the generation; some of these increases may be due to GM technologies and crop protection advances that have been made possible (Yali, 2022).

Microbial Biological Control Agent

Genetic modification or gene editing of plants to increase yield, to protect them against biotic and abiotic stresses and the use of RNAi sprays to regulate gene expression in plants, are among the many biotechnological developments taking place in agriculture (Scheepmaker *et al*., 2016).Another biotechnological approach is the use of microbial biological control agents (MBCAs) for the control of plant disease. Biological control of plant diseases is the suppression of populations of plant pathogens by living organisms (Heimpel and Mills, 2017). Amongst beneficial microorganisms' isolates can be selected which are highly effective against pathogens and can be multiplied on artificial media. Application of such selected and mass-produced antagonists in high densities once or several times during a growing season is called "augmentative biological control" (Heimpel and Mills, 2017; van Lenteren *et al*., 2018). For commercial augmentative biological control of diseases, growers use MBCAs containing living microorganisms that are registered plant protection products produced by biocontrol companies. In some cases, antimicrobial metabolites produced by selected microbial organisms are included in the product, and some products even contain only antimicrobial metabolites without living cells of the antagonist (Glare *et al*., 2012).

Microbial biological control agents (MBCAs) are applied to crops for biological control of plant pathogens where they act via a range of modes of action. Some MBCAs interact with plants by inducing resistance or priming plants without any direct interaction with the targeted pathogen. Other MBCAs

act via nutrient competition or other mechanisms modulating the growth conditions for the pathogen. Antagonists acting through hyperparasitism and antibiosis are directly interfering with the pathogen (Köhl *et al*., 2019). Hyperparasites invade and kill mycelium, spores, and resting structures of fungal pathogens and cells of bacterial pathogens (Ghorbanpour *et al*., 2018). Production of antimicrobial secondary metabolites with inhibiting effects against pathogens is another direct mode of action (Raaijmakers and Mazzola, 2012). Low amounts of in situ secreted secondary metabolites support antagonists to gain a competitive advantage. In some cases, biocontrol agents have been selected which secrete already efficient secondary metabolites into the growth media during mass production that are applied together with or without living cells of antagonists in the biological control product.

Since the nineties of last century research has been performed to improve the efficacy of MBCAs by genetic modification. This can be done by combining traits in one organism or by increasing the persistence of the MBCA or increasing the expression of bioactive components (Walsh *et al.*, 2001).

Soil microbes play a central role in the formation and improvement of soil fertility, farmland material circulation, enhancing stress resistance in plants, resistance towards soil-borne pathogens, degradation and detoxification of heavy metals in soil (Barret *et al*., 2013). Soil microbiota interacts with the plants either directly or indirectly improving the health and fitness of the plants (Baslam *et al*., 2011). Healthy plant-microbial interactions support plants to manage with varying stress conditions and diseases; it improves the exchange of mineral substances such as nitrogen or phosphate, and act as biocontrol agents to prevent pathogen attack in plants (Benckiser and Bamforth, 2011).

In agriculture, microbes are mainly used as inoculants for providing nutrition and protection to plants. Several strains of bacteria can influence the growth, production, and protection of plants. Bacterial strains, which are used to increase plant growth by enhancing the uptake of the nutrient to the plants, are known as plant growth-promoting rhizobacteria. The strains, which protect the plants from the disease-causing pathogens, are used as biocontrol strains. However, the agricultural applications of microbe are not very successful because of their low efficacy in the field. Microbes are therefore modified genetically to improve efficiency. To accomplish this goal, scientists utilize genetic engineering to modify microorganisms and construct new strains with improved properties. For example, genetically modified (GM) Rhizobia and *Azorhizobium* are now used as biofertilizers with efficacy. These bacteria have good survival capability in the field, and have better competitive fitness as compared to the wild-type strain. Besides, some GM bacteria like *Agrobacterium* and *Pseudomonas* have good potential to protect the plants from insects, various disease-causing pathogens, and frost injuries (Madhu *et al*., 2022).

Meeting agriculture productivity without losing the quality of agricultural land is more promising by the application of microbial inoculants. Taking this into consideration, researchers have contributed valuable time for developing and engineering microbial inoculants that could be applied in the agricultural land as biopesticides, bioherbicides, biocontrol agents and biofertilizers. Microbial inoculants are destined to be eco-friendly and sustainable plant nutrient transporters. They have the capability to minimize chemical input impacts and accordingly intensify the quality and quantity of farm products. Use of microbial inoculants reduces the application of chemical fertilizers (Hardoim *et al*., 2015).

Microbial engineering techniques such as gene editing, CRISPR/Cas9, RNAi and others alter the genetic makeup of the microorganisms to improve their beneficial roles towards metabolization of toxic compounds that help plant growth for higher yield and to clean environment (Eltarahony *et al*., 2023). The engineering of wild microbes to produce a potent microbial inoculant offers improved crop productivity, biological control, plant growth, tolerance against biotic and abiotic stresses, nutrient uptake and increased soil fertility.

Biopesticides

Biopesticides are modified or natural microorganisms instead of chemicals. Genetically engineered microbes can be used as environmentally friendly pesticides because it has a lesser negative impact on the ecosystem (Ayilara *et al*., 2023). The use of biopesticides reduces the use of chemical pesticides to manage insects. Pests are the most significant threat to sustainable agriculture because they reduce the plant's yield. Pests include insects, nematodes, plants with parasitic infections, and illnesses (Omran *et al*., 2023). Insect infestations hindered crop cultivation and economic development during the 20th century which employed the development of synthetic pesticides (SPs) (Yadav *et al*., 2023). The industrial sector employs numerous synthetic pesticides, including DDT, aldicarb, fenobucarb, carbofuran, atrazine, deltamethrin (pyrethroids) and simazine (triazines). SPs, in their vast majority, induce neurotoxicity. Notwithstanding their efficiency, these SPs have drawbacks that contravene the tenets of sustainability. It is critical to prioritize the utilization of biopesticides over chemical insecticides. The excessive use of synthetic pesticides can cause negative chronic health impacts such as cancer, damage to the liver, kidneys, lungs, brain and nervous system, birth defects, infertility and other reproductive problems. Agricultural workers are more exposed to pesticides with adverse health outcomes.

Plant Growth Promoting Rhizobacteria (PGPR)

Several microorganisms and rhizobial endophytes like *Rhizobium, Bradyrhizobium, Sinorhizobium, Ochrobactrum, Azorhizobium, Allorhizobium,* and *Mesorhizobium* are commonly used to inoculate enhanced agricultural yield (DeMeyer *et al*., 2015). PGPR aids bioremediation by breaking down xenobiotics and contaminants such as heavy metals and pesticides. The various mechanisms exhibited by PGPR as plant growth enhancers including potassium solubilization,nitrogen fixation (Ahemad and Kibret,2014) siderophores production (Rathore, 2015), phosphate solubilization (Oteino *et al*., 2015), nutrient fixation (Kumar, 2016), phosphate solubilization (Kaur *et al*., 2016), and suppression of plant pathogens (Vocciante *et al*.,2022). PGPRs are also exploiting diverse methods to reduce the effect of stress (Zhang *et al*., 2022c). During drought and high soil salt concentrations, plants experience water stress, osmotic and ionic imbalances, and increases the production of ROS.

Based on the interactions with plants, PGPR can be separated into symbiotic bacteria, whereby they live inside plants and exchange metabolites with them directly, and free-living rhizobacteria, which live outside plant cells (Gray and Smith, 2005). The working mechanisms of PGPR can also be separated into direct and indirect ones. The direct mechanisms are biofertilization, stimulation of root growth, rhizoremediation, and plant stress control. On the other hand, the mechanism of biological control by which rhizobacteria are involved as plant growth promotion indirectly is by reducing the impact of diseases, which include antibiosis,

induction of systemic resistance, and competition for nutrients and niches (Egamberdieva and Lugtenberg, 2014).

Biofertilizer

Biofertilizers consist of efficient genetically modified microbes, organic products and waste parts of plants which gradually increase crop yield by enhancing soil fertility. Microbes inoculated in soil provide resistance against many stresses, like hydrogen ion concentration, high moisture content, salinity, and clay content. GMMs offer better nutrient accessibility to crops and thus increase the growth of plants and crop yield as well. The most significant biofertilizers are symbiotic bacteria like *Rhizobium*, *Bradyrhizobium* and *Sinorhizobium* which forms root nodules and fix nitrogen for plants. Genetically modified biofertilizers are found to be superior in terms of their activity and survival rates. GMM-based biofertilizers supply better nutrient accessibility for crops and supports agricultural practices (Ali *et al.*, 2020)

Biological Control of Plant's Diseases

Microbial engineering may facilitate the establishment of biological control methods for plant diseases. Genetically modified microbes have the potential to produce compounds that exhibit antibacterial or antifungal characteristics, thus assisting plants in their resistance to infections (Kandasamy and Kathirvel, 2023). By reducing the use of fungicides and antibiotics, this strategy contributes to the environmentally sustainable management of agricultural diseases.Many reports are available that indicate the influence of microbes to retard the growth of potent fungal pathogens (Auge *et al*., 2015). The best example of antibiosis is the use of agrocin 84 produced by *Agrobacterium radiobacter* for controlling plant disease. The genetically engineered *Pseudomonas putida* WCS358r strains, produce 2,4-diacetylphloroglucinol (2,4-DAPG) and phenazine, which cause inhibition of wheat pathogens (Bakker *et al*., 2002).

The discovery of genome editing by using the CRISPR/Cas9 technique is a major breakthrough to apply against plant pathogens. Microbial engineering provides a better solution for the growth of a healthy environment and higher agricultural productivity over the existing methods and resolved the challenges worldwide related to development of sustainable agriculture and greener ecosystems (Singh *et al*., 2024).

CONCLUSION

Microorganisms can be genetically altered to add or enhance certain properties, giving them a wider range of application than regular microbial products. This review has shown that microbial engineering and formulations are very important for specific applications especially in medicine, environment, food and agriculture. Genetically engineered microorganisms are being utilized for the production of antibiotics, vaccines and heterologous proteins such as insulin. They are also used to produce new food ingredients, improve or enhance the production of an existing protein/ingredient, or to tailor the characteristics of an existing protein to a new application. Genetically engineered microorganisms are applied in the bioremediation of polluted soils, as bio-detectors to monitor soil pollution, and to enhance nitrogen fixation as well as biopesticides, biofertilizers, plant growth promoting rhizobacteria to mention but a few.

REFERENCES

Ahemad, M., Kibret, M. (2014). Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. *J King Saud Univer Sci*. 26(1):1-20.

Ali, R., Zulaykha, K.D., Sajjad, N. (2020). Genetically Modified Microbes as Biofertilizers. In: Bhat, R.A., Hakeem, K.R. (eds). *Bioremediation and Biotechnology*, 4:275-293

Al-Mariri, A., Tibor, A., Lestrate, P., Mertens, P., De Bolle, X., Letesson, J.J. (2002). Yersinia enterocolitica as a vehicle for a naked DNA vaccine encoding Brucella abortus bacterioferritin or P39 antigen. *Infect. Immun.*70. Https://doi.org/10.1128/IAI.70.4.1915-1923.2002.

Alvarez, B., Fernandez, L. A. Sustainable therapies by engineered bacteria. *Microbial Biotechnology*. 2017; 10(5):1057–1061. Https://doi.org/10.1111/1751-7915.12778

Ari, M.M., Dadgar, L., Elahi, Z., Ghanavati, R., Taheri, B. (2024). Genetically Engineered Microorganisms and Their Impact on Human Health. *Int J Clin Pract*.:6638269. Https://doi.org/10.1155/2024/6638269

Auge, R.M., Toler, H.D., Saxton, A,M. (2015). Arbuscular mycorrhizal symbiosis alters stomatal conductance of host plants more under drought than under amply watered conditions: a meta-analysis. *Mycorrhiza.* 25(1):13-24.

Ayilara, M.S., Adeleke, B.S., Akinola, S.A, *et al*. Biopesticides as a promising alternative to synthetic pesticides: A case for microbial pesticides, phytopesticides, and nanobiopesticides. *Front Microbiol.*2023; 14:1040901

Bakker, P.A., Glandorf, D.C., Viebahn, M., Ouwens T.W., Smith, E., Leeflang, P…van Loon, L.C. (2002). Effects of *Pseudomonas putida* modified to produce phenazine-1-carboxylic acid and 2, 4-diacetylphloroglucinol on the microflora of field grown wheat. *Antonie Van Leeuwenhoek.* 81(1-4):617-24.

Barnett, J. A. (2003). Beginnings of microbiology and biochemistry: The contribution of yeast research. *Microbiology* 149 (3):557–67. Https://doi.org/10.1099/mic.0.26089-0.

Barret, M., Tan, H., Egan, F., Morrissey, J. P., Reen, J., & O'Gara, F. (2013). Exploiting New Systems-Based Strategies to Elucidate Plant–Bacterial Interactions in the Rhizosphere. *Molecular microbial ecology of the rhizosphere*, *1*, 57-68.

Baslam, M., Garmendia, I., Goicoechea, N. (2011). Arbuscular mycorrhizal fungi (AMF) improved growth and nutritional quality of greenhouse-grown lettuce. *J. Agric. Food Chem.*; 59(10):5504–5515 Https://doi.org/10.1021/jf200501c.

Benckiser, G., and Bamforth, S.S. (2011). Role of pathogens, signal recalcitrance, and organisms shifting for ecosystem recuperation. A review. *Agronomy Sust. Developm.* 31(1):205–215. Https://doi.org/10.1051/agro/2010024.

Binda, S., Hill, C., Johansen, E., Obis, D., Pot, B., Sanders, M.E., Ouwehand, A. (2020). Criteria to Qualify Microorganisms as "Probiotic" in Foods and Dietary

Supplements. *Front Microbiol*; 11:1662. Https://doi.org/10.3389/fmicb.2020.01662.

BOC Sciences (2026). https://www.bocsci.com/resources/genetically-engineered-vaccines-advantages-application-and-examples.html

Brochado, A. R., Matos, C., Møller, B.L., Hansen, J., Mortensen, U.H. and Patil, K.R. (2010). Improved vanillin production in baker's yeast through in silico design. *Microbial Cell Factories* 9 (1):84. doi: https://doi.org/10.1186/1475-2859-9-84

Chang, M., Chu, X., Lv, J., Li, Q., Tian, J.;Wu, N. (2016). Improving the thermostability of acidic pullulanase from bacillus naganoensis by rational design. PLoS ONE, 11, 1–12.

Chen, K., Zhu, Y., Zhang, Y., Hamza, T., Yu, H, Saint Fleur, A., Feng, H. (2020). A probiotic yeast-based immunotherapy against Clostridioides difficile infection. *Sci Transl Med*; 12:567.

Chen Z., Guo L., Zhang Y., Walzem, R.L., Pendergast, J.S., Printz, R.L Davies, S.S. (2014). Incorporation of therapeutically modified bacteria into gut microbiota inhibits obesity. *Journal of Clinical Investigation*, 124(8):3391–3406. Https://doi.org/10.1172/JCI72517

Chen, H., Ji, P.C., Qi, Y.H., Chen, S.J., Wang. C.Y., Yang, Y.J…Zhao X.(2023). Inactivation of *Pseudomonas aeruginosa* biofilms by thymoquinone in combination with nisin. *Front Microbiol*; 13:1029412.

Chowdhury, M.Y., Li, R., Kim, J.H., Park, M.E., Kim T.H., Pathinayake, P…Hong S.P. (2014). Mucosal vaccination with recombinant *Lactobacillus casei*-displayed CTA1-conjugated consensus matrix protein-2 (sM2) induces broad protection against divergent influenza subtypes in BALB/c mice. *PLoS ONE*. 2014; 9:e94051.

Citovsky V., Kozlovsky S.V., Lacroix B., Zaltsman A., Dafny-Yelin, M., Vyas S Tzfira, T. (2007). "Biological systems of the host cell involved in *Agrobacterium*infection,"*Cellular Microbiology*, vol. 9, no. 1, pp. 9–20, 2007.

De Meyer, S.E., De Beuf, K., Vekeman, B., Willems, A.A. (2015). Large diversity of non-rhizobial endophytes found in legume root nodules in Flanders (Belgium). *Soil Biol Biochem.* 83:1-11.

Dosoky, N. S., May-Zhang, L.S., Davies S. S. (2020). Engineering the gut microbiota to treat chronic diseases. *Applied Microbiology and Biotechnology*, 104(18):7657–7671. Https://doi.org/10.1007/s00253-020-10771-0.

Duan, F.F., Liu, J.H., March, J.C. (2015). Engineered commensal bacteria reprogram intestinal cells into glucose-responsive insulin-secreting cells for the treatment of diabetes. *Diabetes*, 64(5):1794–1803. Https://doi.org/10.2337/db14-0635.

Egamberdieva, D. and Lugtenberg, B. (2014). *Use of Microbes for the Alleviation of Soil Stresses*. Volume 1. Springer; New York, NY, USA: Use of Plant Growth-Promoting Rhizobacteria to Alleviate Salinity Stress in Plants; pp. 73–96.

Egeland, T.A., Gaustad J.V., Galappathi K., Rofstad E.K. (2011). Magnetic resonance imaging of tumor necrosis. *Acta Oncologica*, 50(3):427–434. Https://doi.org/10.3109/0284186x.2010.526633

Eltarahony, M., Ibrahim, E., Hegazy, G., Sabry, A. (2023). Microbial Remediation of Mercury: An Overview. *Mercury Toxicity: Challenges and Solutions*, 201-234.

Fontana, C., Fadda, S., Cocconcelli, P.S., &Vignolo, G., (2012). "*Lactic acid bacteria in meat fermentations". Lactic Acid Bacteria–Microbiological and Functional Aspects*, 4th Ed. CRC Press, Taylor & Francis, Boca Raton, London, New York, 247-264.

Fridovich-Keil, J. L. and Diaz, J. M. (2024). *Genetically modified organism. Encyclopedia Britannica*. https://www.britannica.com/science/genetically-modified-organism.

Gagnon, K. (2009). Chapter 9 - Measuring Electroneutral Chloride-Dependent Ion Fluxes in Mammalian Cells and in Heterologous Expression Systems. in Physiology and Pathology of Chloride Transporters and Channels in the Nervous System by F. Javier Alvarez-Leefmans and Eric Delpire. https://doi.org/10.1016/B978-0-12-374373-2.00009-1

Geldart, K.G., Kommineni, S., Forbes, M., Hayward, M., Dunny, G.M., Salzman, N.H, *et al*. (2018). Engineered E. coli Nissle 1917 for the reduction of vancomycin-resistant Enterococcus in the intestinal tract. *Bioeng Transl Med*.3 (3):197–208.

Ghanavati, R., Akbari, A., Mohammadi, F., *et al*. (2020). Lactobacillus species inhibitory effect on colorectal cancer progression through modulating the Wnt/β-catenin signaling pathway. Molecular and Cellular Biochemistry, 470(1-2):1–13. Https://doi.org/10.1007/s11010-020-03740-8.

Ghorbanpour, M., Omidvari, M., Abbaszadeh-Dahaji, P., Omidvar, R., and Kariman, K. (2018). Mechanisms underlying the protective effects of beneficial fungi against plant diseases. *Biol. Control* 117, 147–157. Https://doi.org/10.1016/j.biocontrol.2017.11.006

Ghosh, A.K., Devenport, M., Jethwaney, D., *et al*. (2009). Malaria parasite invasion of the mosquito salivary gland requires interaction between the Plasmodium TRAP and the Anopheles saglin proteins. PLoSPathogens, 5(1) Https://doi.org/10.1371/journal.ppat.1000265.e1000265.

Glare, T., Caradus, J., Gelernter, W., Jackson, T., Keyhani, N., Köhl, J…Stewart, A. (2012). Have biopesticides come of age? *Trends Biotechnol.* 30, 250–258. Https://doi.org/10.1016/j.tibtech.2012.01.003.

Glazer, A.N. and Nikaido, H. (2007). MICROBIAL BIOTECHNOLOGY: Fundamentals of Applied Microbiology, Second Edition. Cambridge University Press, New York.

Gorlach, K. (1994). Problems in the introduction of genetically engineered microorganisms into the environment. *Acta Microbiologica Polonica*; 43(2):121–131.

Grangette, C., Müller-Alouf, H., Goudercourt, D., Geoffroy, M.C., Turneer, M., Mercenier A. (2001). Mucosal immune responses and protection against tetanus toxin after intranasal immunization with recombinant Lactobacillus plantarum. *Infect. Immun*.69 Https://doi.org/10.1128/IAI.69.3.1547-1553.2001.

Gray E.J., Smith D.L. Intracellular and extracellular PGPR: Commonalities and distinctions in the plant-bacterium signaling processes. *Soil Biol. Biochem.* 2005; 37:395–412. Https://doi.org/10.1016/j.soilbio.2004.08.030.

Hanlon, P., &Sewalt, V. (2020). GEMs: genetically engineered microorganisms and the regulatory oversight of their uses in modern food production. *Critical Reviews in Food Science and Nutrition*, *61*(6), 959–970. https://doi.org/10.1080/10408398.2020.1749026

Hardoim P.R., van Overbeek L.S., Berg G., Pirttilä A.M., Compant S., Campisano A., Döring M., SessitschA.(2015). The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiol. Mol. Biol. Rev*; **79**(3):293–320. Https://doi.org/10.1128/MMBR.00050-14.

Hashimoto T., J. Hashimoto, K. Teruya *et al*., "Biosynthesis of versipelostatin: identification of an enzyme-catalyzed [4+2]-cycloaddition required for macrocyclization of spirotetronate-containing polyketides," *Journal of the American Chemical Society*, vol. 137, no. 2, pp. 572–575, 2015.

Heimpel, G. E. and Mills, N. (2017). *Biological Control - Ecology and Applications*. Cambridge: Cambridge University Press.
Hjort, C. (2007). Industrial Enzyle Production for Food Applications;Woodhead Publishing Limitedp: Cambridge, UK.

Hwang, I.Y., Koh, E., Wong, A., March, J.C., Bentley, W.E., Lee, Y.S., *et al*. (2017). Engineered probiotic *Escherichia coli* can eliminate and prevent Pseudomonas aeruginosa gut infection in animal models. Nat Commun, 8:15028

Kandasamy, G.D., and Kathirvel, P. (2023). Insights into bacterial endophytic diversity and isolation with a focus on their potential applications–A review. *Microbiol Res*., 266:127256

Kaur H, Kaur J, Gera R. Plant Growth Promoting Rhizobacteria: A Boon to Agriculture. *Int J Cell Sci Biotechnol*. 2016; 5:317-322.

Köhl J., Kolnaar R. and Ravensberg, J.W. (2019). Mode of Action of Microbial Biological Control Agents against Plant Diseases: Relevance beyond Efficacy. . Front. Plant Sci., Sec. Plant Pathogen Interactions,Volume 10 - https://doi.org/10.3389/fpls.2019.00845

Kumar A. (2016). Phosphate Solubilizing Bacteria in Agriculture Biotechnology: Diversity, Mechanism and Their Role in Plant Growth and Crop Yield. *Int J Adv Res*. 4(4):116-124.

Lanigan, T. M., Kopera, H. C., & Saunders, T. L. (2020). Principles of Genetic Engineering. *Genes*, *11*(3), 291. https://doi.org/10.3390/genes11030291.

Li D., Tang, Y., Lin, J. and Cai W. (2017). "Methods for genetic transformation of :lamentous fungi," *Microbial Cell Factories*, vol. 16, no. 1, p. 168.

Liang A., Riaz H., Dong F., Luo X., Yu X., Han Y., *et al*. (2014).Evaluation of efficacy, biodistribution and safety of antibiotic-free plasmid encoding somatostatin genes delivered by attenuated Salmonella enterica serovar Choleraesuis. Vaccine.32 Https://doi.org/10.1016/j.vaccine.2014.01.026.

Liu, X., Kokare, C. (2017). Microbial Enzymes of Use in Industry. In Biotechnology of Microbial Enzymes; Elsevier: Amsterdam, The Netherlands.

Lloren, K.K.S., Senevirathne, A, Lee, J.H. (2024). Advancing vaccine technology through the manipulation of pathogenic and commensal bacteria. Mater Today Bio.16; 29:101349 Https://doi.org/10.1016/j.mtbio.2024.101349.

Lubkowicz, D., Ho, C.L., Hwang, I.Y., Yew, W.S., Lee, Y.S., Chang, M.W. (2018). Reprogramming Probiotic *Lactobacillusreuteri* as a Biosensor for Staphylococcus aureus Derived AIP-I Detection. ACS Synth Biol; 7 (5):1229–37.

Madhu, Amandeep Kaur, Santosh Kumar Upadhyay, Chapter 15 - Agricultural applications of engineered microbes, Editor(s): Swati Joshi, Ashok Pandey, RanjnaSirohi, Sung Hoon Park, Current Developments in Biotechnology and Bioengineering, Elsevier, 2022, Pages 363-375, ISBN 9780323885041, https://doi.org/10.1016/B978-0-323-88504-1.00008-X.

Mahdizade, A.M., Dadgar, L., Elahi, Z., Ghanavati, R., Taheri, B. (2024). Genetically Engineered Microorganisms and Their Impact on Human Health. *Int J Clin Pract*; 6638269. Https://doi.org/10.1155/2024/6638269.

Mao, N., Cubillos-Ruiz, A., Cameron, D.E. and Collins, J.J. (2018). Probiotic strains detect and suppress cholera in mice. *Sci Transl Med*; 10:445.

Mazhar, S., Yasmeen, R.., Chaudhry, A., Summia, K., Ibrar, M., Amjad, S., & Ali, E. (2022). Role of Microbes in Modern Food Industry. *International Journal of Innovations in Science & Technology*, *4*(1), 65–77. Retrieved from https://journal.50sea.com/index.php/IJIST/article/view/82

McIntyre, L., Hudson, J. A., Billington, C., & Withers, H., (2012). "Biocontrol of foodborne bacteria". In Novel technologies in food science (pp. 183-204). Springer, New York, NY.

Mokoena, M.P. (2017). Lactic acid bacteria and their bacteriocins: classification, biosynthesis and applications against uropathogens: a mini-review. *Molecules*; 22(8):1255. Https://doi.org/10.3390/molecules22081255.

Montefiori D. C. (2005). Evaluating neutralizing antibodies against HIV, SIV, and SHIV in luciferase reporter gene assays. *Current Protocols in Immunology*, 12(1):1–17. Https://doi.org/10.1002/0471142735.im1211s64.

National Research Council (US) Committee on Identifying and Assessing Unintended Effects of Genetically Engineered Foods on Human Health. (2004). *Safety of Genetically Engineered Foods: Approaches to Assessing Unintended Health Effects*. National Academies Press (US). https://doi.org/10.17226/10977

Nemunaitis J., Meyers T., Senzer N., Cunningham, C., West, H., Vallieres E., Cheever, M.A. (2006). Phase I trial of sequential administration of recombinant DNA and adenovirus expressing L523S protein in early stage non-small-cell lung cancer. *Molecular Therapy*, 13(6):1185–1191. Https://doi.org/10.1016/j.ymthe.2006.01.013.

Olempska-Beer, Z.S., Merker, R.I., Ditto, M.D., DiNovi, M.J. (2006). Food-processing enzymes from recombinant microorganisms—A review. *Regul. Toxicol. Pharmacol*. 45, 144–158.

Omran, E.S.E., Mahmoud, M.F., Abd El-Nabi, HM. (2023). Eco-Friendly Nematodes: Bioindicators for Promoting Sustainable Soil Health. *Nematode-Plant Interactions and Controlling Infection*. 187-213.

Oteino, N., Lally, R.D., Kiwanuka, S., Lloyd, A., Ryan, D., Germaine, K.J. (2015). Plant Growth Promotion Induced by Phosphate Solubilizing Endo-phytic *Pseudomonas* Isolates. *Front Microbiol*, 6:745.

Pan, H., Li, L., Pang, G., Han, C., Liu, B., Zhang, Y.,…Wang, H. (2021). Engineered NIR light-responsive bacteria as anti-tumor agent for targeted and precise cancer therapy. Chemical Engineering Journal, 426 Https://doi.org/10.1016/j.cej.2021.130842.130842

Papagianni, M. and Avramidis, N. (2012). Engineering the central pathways in *Lactococcuslactis*: functional expression of the phosphofructokinase (pfk) and alternative oxidase (aox1) genes from *Aspergillus niger* in *Lactococcus lactis* facilitates improved carbon conversion rates under oxidizing conditions. *Enzyme Microb Technol*; 51(3):125–30.

Parisa, A., Roya, G., Mahdi, R., Shabnam, R., Maryam, E., Malihe, T. (2020). Anti-cancer effects of Bifidobacterium species in colon cancer cells and a mouse model of carcinogenesis. *PLoSOne*, 15(5) Https://doi.org/10.1371/journal.pone.0232930.e0232930.

Philippe, R. N., De Mey M., Anderson, J. and Ajikumar, P.K. (2014). Biotechnological production of natural zero-calorie sweeteners. *Current Opinion in Biotechnology* 26:155–161. doi: https://doi.org/10.1016/j.copbio.2014.01.004

Piñero-Lambea C., Ruano-Gallego D., Fernández L. Á. (2015). Engineered bacteria as therapeutic agents. *Current Opinion in Biotechnology*, 35:94–102. Https://doi.org/10.1016/j.copbio.2015.05.004.

Portieles, R., Xu, H., Chen, F., Gao, J., Du, L., Gao, X…Hidalgo O. (2023). Bioengineering of a *Lactococcuslactis* subsp. *lactis* strain enhances nisin production and bioactivity. PLoS One. ;18(4):e0281175. doi: 10.1371/journal.pone.0281175. PMID: 37036850.

Raaijmakers, J. M., van der Sluis, I., Koster, M., Bakker, P. A. H. M., Weisbeek, P. J., and Schippers, B. (1995). Utilization of heterologous siderophores and rhizosphere competence of fluorescent *Pseudomonas* spp. *Can. J. Microbiol.* 41, 126–135. Https://doi.org/10.1139/m95-017

Rafeeq H, Afsheen N, Rafique S, Arshad A, Intisar M, Hussain A, Bilal M, Iqbal HMN.(2023). Genetically engineered microorganisms for environmental remediation. *Chemosphere*. 310:136751. doi: 10.1016/j.chemosphere.2022.136751. Epub 2022 Oct 6. PMID: 36209847.

Ramesh, N., Ge Y., Ennist, D.L., *et al*. (2006). CG0070, a conditionally replicating granulocyte macrophage colony-stimulating factor–armed oncolytic adenovirus for the treatment of bladder cancer. *Clinical Cancer Research*, 12(1):305–313. Https://doi.org/10.1158/1078-0432.ccr-05-1059.

Rathore, P. (2015). A Review on Approaches to Develop Plant Growth Promoting Rhizobacteria. *Int J Recent Sci Res*., 5(2):403-407.

Rebello, S., Nathan, V.K., Sindhu, R., Binod, P., Awasthi, M.K., and Pandey, A. (2021). Bioengineered microbes for soil health restoration: present status and future. *Bioengineered*; 12(2):12839-12853. Https://doi.org/10.1080/21655979.2021.2004645.

Robinson, P.K. (2015). Enzymes: Principles and biotechnological applications. *Essays Biochem*, 59, 1–41.

Röder J., Felgner P., Hensel M. (2021).Comprehensive single cell analyses of the nutritional environment of intracellular Salmonella enterica. Front. *Cell. Infect. Microbiol*.11 Https://doi.org/10.3389/fcimb.2021.624650.

Rodríguez de Fonseca F., Navarro M., Gómez R., *et al*. (2001). An anorexic lipid mediator regulated by feeding. *Nature*, 414(6860):209–212. Https://doi.org/10.1038/35102582.

Scheepmaker, J.W.A., Hogervorst, P.A.M. and Glandorf, D.C.M. (2016). Future introductions of genetically modified microbial biocontrol agents in the EU. Are current EU legislation and risk assessment fit for purpose? RIVM Letter report 2016-0057 https://www.rivm.nl/bibliotheek/rapporten/2016-0057.pdf

Schoen C., Stritzker J., Goebel W., Pilgrim S. (2004). Bacteria as DNA vaccine carriers for genetic immunization. International Journal of Medical Microbiology. 294 Https://doi.org/10.1016/j.ijmm.2004.03.001.

Sedighi M., Zahedi Bialvaei A., Hamblin M. R., *et al*. (2019). Therapeutic bacteria to combat cancer; current advances, challenges, and opportunities. *Cancer Medicine*, 8(6):3167–3181 Https://doi.org/10.1002/cam4.2148.

Sedighi, M., Bialvaei A.Z., Hamblin M. R., Ohadi E., Asadi A., Halajzadeh, M…Kouhsari E. (2019). Therapeutic bacteria to combat cancer; current advances, challenges, and opportunities. *Cancer Medicine*; 8(6):3167–3181. Https://doi.org/10.1002/cam4.2148

Singh, P., Singh, R., Singh, S., Chauhan, RS, Bala, S., Pathak, N Tripathi, M. (2024).Microbial engineering for a Greener Ecosystem and Agriculture: Recent Advances and

Challenges. *J Pure Appl Microbiol.* 18(2):797-807. https://doi.org/10.22207/JPAM.18.2.23

Singh, R., Kumar, M., Mittal, A., Kumar, P. (2016). Microbial enzymes: Industrial progress in 21st century. *3 Biotech*, 6, 1–15.

Steidler, L., Hans, W., Schotte, L., Neirynck, S., Obermeier, F., Falk, W., Fiers, W., Remaut, E. (1979). Treatment of murine colitis by Lactococcus lactis secreting interleukin-10, *Science*, 289 (2000), pp. 1352-1355.

Suez, J., Zmora, N., Segal, E., Elinav, E. (2019). The pros, cons, and many unknowns of probiotics. *Nat Med.;* 25(5):716–729. Https://doi.org/10.1038/s41591-019-0439-x.

Trono, D. (2019). Recombinant Enzymes in the Food and Pharmaceutical Industries; Elsevier B.V.: Amsterdam, The Netherlands.

Van Lenteren, J. C., Bolckmans, K., Köhl, J., Ravensberg, W. J., and Urbaneja, A. (2018). Biological control using invertebrates and microorganisms: plenty of new opportunities. *BioControl* 63, 39–59. Https://doi.org/10.1007/s10526-017-9801-4

Vermeiren, L., Devlieghere, F., Vandekinderen, I., Rajtak, U., &Debevere, J. (2006). "The sensory acceptability of cooked meat products treated with a protective culture depends on glucose content and buffering capacity: A case study with *Lactobacillus sakei* 10A". *Meat Science*, 74(3): 532-545

Vieille, C., Zeikus, G.J. (2001). Hyperthermophilic Enzymes. Microbiol. Mol. Biol. Rev., 65, 1–43.

Vocciante, M., Grifoni, M., Fusini, D., Petruzzelli, G., Franchi, E. (2022).The role of plant growth-promoting rhizobacteria (PGPR) in mitigating plant's environmental stresses. *Appl Sci.*; 12:1231.

Waksman, G. (2019)"From conjugation to T4S systems in Gramnegative bacteria: a mechanistic biology perspective," *EMBO Reports*, vol. 20, no. 2, Article ID e47012.

Walsh, U.F., Morrissey, J.P., and O'Gara, F. (2001). *Pseudomonas* for biocontrol of phytopathogens: from functional genomics to commercial exploitation. Current Opinion in Biotechnology, 12(3): p. 289-295.

Wei, Y., Yang, F., Wu, Q., Gao, J., Liu, W., Liu, C., Suwal, S. (2018). Protective effects of bifidobacterial strains against toxigenic *Clostridium difficile*. *Front. Microbiol.* 9 Https://doi.org/10.3389/fmicb.2018.00888

Wiese C., Grieskamp T., Airik R., Mommersteeg, MTM. Gardiwal, A., Vries, C.G. Christoffels, V.M. (2009). Formation of the sinus node head and differentiation of sinus node myocardium are independently regulated by Tbx18 and Tbx3. *Circulation Research*, 104(3):388–397. Https://doi.org/10.1161/circresaha.108.187062

Wright, A., and Bruce, A. (2003). Genetically modified microorganisms and their potential effects on human health and nutrition. Trends in Food Science & Technology 14, 264–276

Xu, F., Hong, M., Ulmer, J.B. (2003). Immunogenicity of an HIV-1 gag DNA vaccine carried by attenuated Shigella. *Vaccine*.21. Https://doi.org/10.1016/S0264-410X(02)00573-X

Yadav, S.P.S., Adhikari, R., Bhatta, D., Paudel, A., Subedi, S., Shreshtha, S. & Shreshtha, J. (2023). Initiatives for biodiversity conservation and utilization in crop protection: A strategy for sustainable crop production. *Biodiversity and Conservation*, 32(14):4573-4595

Yali, W. (2022). Application of Genetically Modified Organism (GMO) Crop Technology and Its Implications in Modern Agriculture. *International Journal of Applied Agricultural Sciences*. Vol. 8, No. 1, pp. 1-8. Https://doi.org/10.11648/j.ijaas.20220801.11

Zhang H., Zhao, H., Zhao, Y., Sui, L., Li, F., Zhang, H., & Li, Y. (2022a). Auxotrophic lactobacillus expressing porcine rotavirus VP4 constructed using CRISPR-Cas9D10A system induces effective immunity in mice. Vaccines (Basel);10 Https://doi.org/10.3390/vaccines10091510

Zhang K., Tang Y., Chen Q., Liu Y. (2022b).The screening of therapeutic peptides for anti-inflammation through phage display technology. *International Journal of Molecular Sciences*;23(15):p. 8554. Https://doi.org/10.3390/ijms23158554

Zhang, M., Liu, L., Chen, C., Zhao, Y., Pang, C., Chen, M. (2022c). Heterologous expression of a *Fraxinus velutina* SnRK2 gene in *Arabidopsis* increases salt tolerance by modifying root development and ion homeostasis. *Plant Cell Rep*.41(9):1895-1906

Zhang, Y. P., Sun J., and Ma, Y. (2017). Biomanufacturing: History and perspective. *Journal of Industrial Microbiology & Biotechnology*, 44 (4-5):773–784. Https://doi.org/10.1007/s10295-016-1863-2