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Review Paper

Recent Advances in Genetically Engineered Microorganisms and their Risks: A Review

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***Abstract*—Genetically engineered microorganisms have applications in various domains, such as agriculture, bioscience, healthcare, life sciences, and research. The novel methods of the system Clustered Regularly Interspaced Short Palindromic Repeats associated with protein 9, which originates from archaeal and bacterial immune systems and allows significant improvements to modified strains of microorganisms, represented a major innovation in industrial biotechnology. The rapid advancement of genetically engineered microorganisms has shown potential for bioremediation, food enzyme production, probiotics, and pesticides. Recently, engineered microbes have been used in several industries, like dairy, pharmaceuticals, biotech, and agrochemicals. Modified microorganisms used as biosensors are improved with reporter genes that induce their expression depending on the nature and concentration of the compound of interest to monitor environmental pollution. Genetically engineered microorganisms have been considered a threat to the environment, animals, and human health. Insertion of a single gene into different cells can result in diverse outcomes, and the general pattern of gene expression can be changed. More advanced and better techniques should be developed and applied in the genetic engineering of microbes to minimize risks.**

Keywords*— *Bioremediation, Biopesticide, CRISPR, Genetically engineered microorganisms

I. INTRODUCTION

Microorganism have an important role on environmentally, economically and socially. They have advantage to perform a key role and act as prominent engineers in overriding all ecological transformations [1]. Genetically engineered microorganism is those microorganisms whose genetic material has been already changed by different genetic engineering techniques by natural or artificial genetic exchange between microorganisms [2]. Recombinant living organisms obtained by recombinant DNA techniques or by natural genetic material exchange between organisms [3]. The first organisms genetically modified in the laboratory were bacteria, because of the relative ease of altering their chromosomes. Bacteria are inexpensive, easy to grow, reproduce rapidly and can be stored at low temperature. This ease made them important tools for the creation of other genetically modified organisms [4]. Genes and other genetic information from a wide range of organisms can be added to a plasmid and introduced into bacteria for storage and modification [5]. Isolated gene can be stored inside the bacteria to provide an unlimited supply for research. A large number of custom plasmids make manipulating DNA extracted from bacteria relatively easy [6].

Recently biotechnology has allowed the movement of genetic material across distinct species, those impossible with the outdated breeding approaches. Genetic engineering techniques enable scientists to find individual genes that governor particular characteristics, separate them from the original source, and transfer them directly into the cells of an animal, plant, bacterium or virus [7]. Genetic modification has been enhanced the consumption and removal of dangerous undesirable wildernesses under laboratory conditions by creating genetically modified organisms [8]. Environmental applications of microorganisms are wide ranging and diverse from bioremediation, biopesticides, nitrogen fixation, plant growth promoter, to biocontrol of plant diseases, and other such agricultural practices. The practical application of recombinant DNA techniques has shown the potential for genetically improved microorganisms to be used as soil or seed inoculants [9].

When genetically engineered microbes introduced into the environment, they could have unintended environmental consequences and may play more pronounced ecological roles than the wild types [10]. Genetically improved microorganisms are able to reproduce and establish themselves as persistent populations and may have long-term effects on biological communities and natural ecologies [11]. The extraordinary successes in biochemistry and molecular biology over the past decades have led to extensive use of GEMs (Genetically engineered microorganisms) in the production of medical and food substances [12].

Genetically engineered microbes are advancing food production by increasing efficiency, reducing waste and resource requirements and ultimately enabling beneficial innovations such as the cost-effective fortification of food with essential nutrients, vitamins, and amino acids, and delivery of tailored enzymes to achieve unique food processing capabilities [13].Genetic modification of microbial strains usually acts to increase the production of preferred compounds or limit the release of unwanted metabolic yields. The possibility of introducing genetically modified organisms (GMOs) and genetically modified microorganisms (GMMs) into the food industry represents an interesting solution for meeting the market demands [14]. DNA modification may not be limited only to the particular characteristics of the replaced gene, therefore it is important to ensure that when these organisms are released into nature they do not harm the environment or human health. Such concerns have led to wide-ranging interests in the theme of risk assessment in the release of GMOs [1].

Objective of this review is mainly to illustrate current advances in genetically engineered microorganisms application as well as risk that associated with transgenic microbes on environment, health of animal and human.

II. Historical Background Of Genetically Engineered Microorganisms

Human activity has been linked with microorganisms for many centuries. One of the ancient known was food preservation methods is fermentation, this fermentation process has been used for conserving foods, making different food products, pharmaceutical manufacturing and environmental management was those principal [15]. This kind of advances already started early in the history of humanity with the use of microorganisms [16].

The fermentation processes for gaining those food products are very complex and usually involve many types of microorganisms, like yeast, lactic acid bacteria (LAB), and fungus [17]. Then industrial revolution comes and change need for processed food, food products containing additives with high nutritional value and with minimal health risks [16]. The emergence and development of genetic engineering through past few decades improves novel properties that have high potential for use in industry of food, chemical, and pharmaceutical as well as biotechnology and medicine [18].

Genetically engineered microorganism revolutionize the food industry by generating products of superior quality with more pronounced tastes and smells and pleasant textures. However, despite the huge number of research studies concerning the amelioration of microorganisms through genetic engineering techniques, at present only a few GMM strains are commercially used or pending approval. Therefore attempts are being made to use alternative techniques for the genetic and metabolic amelioration of food microorganisms [19].

In the early 1970s, microorganisms, E. coli, were used at the pole position of molecular biology research, ensuing in the beginning of recombinant DNA technology [20]. In 1982 FDA (Food and Drug Authority) approved first recombinant protein, human insulin, produced by genetically engineered microorganism as a drug for diabetes [21]. Since then many recombinant proteins have been engineered and expressed in microorganisms and approved for use as pharmaceuticals [22].

Recombinant DNA technology presents a significant advances in genetically modification of organism for economic importance [23]. Genetic modification of microbes is much easier compared to multicellular organisms. Many microorganisms are single cell organisms, in laboratory those microorganism can be easily grown in large quantities and their DNA can easily introduced [24]. Genetically engineered microorganism recently used in the pharmaceutical and biotech organizations for production of antibiotics and proteins. Different proteins were cloned using modified microorganism for production of human insulin, development hormone, and hepatitis B immunization [25]. In general less complicated compared with multicellular organisms, facilitating targeted genetic manipulations [26].

III. Basic Steps in Genetic Engineering of Microorganisms

Genetic engineering is a modern technology, which allows to design microorganisms capable of degrading, sensing, specific contaminants or products [106]. Genetic engineering creates artificial mixture of genes those not occur composed in nature [27]. The most often methods used comprise engineering through single genes or operons, pathway construction and alternations of sequences of existing genes [28].

The first step is to separate and isolate DNA of our interest to identify the genes or genes that we are interested in, the DNA of target. The green fluorescent protein (GFP) commonly used as an expression marker in molecular techniques. In cloning a gene it is helpful to use a cloning vector, typically a plasmid or virus, capable of independent Replication that will stably carry the target DNA from one location to another [29].

The second step is to cut isolated DNA with restriction endonucleases, once the target and vector DNA have been identified, both types of DNA are cut using Restriction endonucleases [30]. The enzymes are widespread in both bacteria and archaea, with each enzyme recognizing a specific inverted repeat sequence that is palindromic [31]. The gene is separated by using restriction enzymes to cut the DNA into fragments or PCR to amplify up the gene segment. Gel electrophoresis is used to extract the segment. For the known DNA sequence, but no copies of the gene are available, it can also be artificially synthesized [32, 33].

The third step is combining target and vector DNA, Restriction endonuclease are used to cleave for both types of DNA. DNA ligase is used to combine those DNA together [27]. Once the gene of our interest is ligated into a plasmid then introduced into a bacterium. The plasmid is replicated when the bacteria divide or multiply, ensuring unlimited copies of the gene are available [34]. This results in the recombinant DNA formation. Plasmids are commonly used as cloning vectors in genetic engineering to multiply or express particular genes [35]. Vector is a genetic molecule for transfer of a new genetic information into another cells, where it replicates independently of their chromosomal DNA [10, 31].

The fourth step is introducing recombined DNA into host cell. The target DNA that has been stably combined with vector

DNA or the recombinant DNA must be introduced into a host cell, to obtain the target genes to be expressed [36]. Recently, with phage vectors, an in vitro packaging method has been developed to overcome the need for E. coli cells to take up free DNA. Techniques like transduction, electroporation, transfection and transformation was used for getting DNA from cells and molecular cloning process significantly detectable through the experimental methods (Fig. 10) [37].

The fifth step is selection of microbial cells containing the needed recombinant vectors and then growth of the transformed microorganisms [32].

The last Step is Expression of the gene to obtain the desired product [33, 37].

The major object of genetic manipulations was done mainly from genus Pseudomonas. There are everywhere occupants of many environment and recognized as efficient degraders of many toxic and lethal substances. Therefore, such microorganisms are the main source of catabolic genes for genetic engineering [38].

IV. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) Technology for Genetic Engineering of Microbes

The advances of the system clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) embodied a major innovation in application of biotechnology which originates from archaeal and bacterial immune systems [39]. The Cas9 endonuclease introduces double-stranded breaks into the target DNA. Using endogenous repair pathways, the genomic DNA can either be altered by introducing mutations or by introducing a specific donor sequence. This is mediated by the non-homologous end-joining DNA repair mechanism or by the homologous repair system [40].

CRISPR/Cas9 is tremendously orthogonal and multipurpose system has stimulated research in the fields of metabolic engineering, systems biology and synthetic biology [41]. The CRISPR/Cas9 technology consists of different toolbox includes CRISPR/Cas9 editing, activation, interference, and CRISPR-mediated protein imaging. These basic tools can be used to classic the genome and program the wanted gene expression, stonework the way for efficient genomic and transcriptomic manipulations to improve the productivity of microbes. The method has been successfully applied to a number of industrially important microorganisms including bacteria, yeast, and filamentous fungi [42].

Selection of recombinant strains using CRISPR/Cas9 system used as an alternative to antimicrobial resistance genes. Using CRISPR/Cas9 technique, the extracellular pullulanase production were enhanced in the B. subtilis strain WS5. Since proteases can degrade heterologous enzymes, such as for pullulanase, genes related to the protease production were disrupted [43].

CRISPR/Cas9 has also been used for the modification of prokaryotes. One of the best example is, an industrial Penicillium subrubescens strain has been developed. Because, the ku70 gene was deleted, which participated in the non-homologous end joining (NHEJ) DNA reparation mechanism. With this deletion, the homologous repair system is favored, and the insertion of a specific and desired DNA sequence into the cleavage site is favored [44].

Genetically modified microbes have provided an attractive way intended for biosynthesis of numerous chemicals from renewable resources. CRISPR-Cas methods have assisted as influential appliances for making cell factories with required properties by working on nucleic acids quickly and efficiently [45].

V. Genetically Engineered Microorganisms for Bioremediation

Ecological community contains microorganisms that have complex relation with other organism. Co-metabolism is the main mechanism of degradation of pollutants. Moreover, synergism is common among microorganisms for pollutant degradation [46].

Recombinant DNA and RNA technologies have been used for engineering microbes, for utilizing for the removal of heavy metals and toxic substance from contaminated sites. Transgenic plants can also mobilize or destroy chlorinated solvent, xenobiotic molecules, explosives and phenolic substances (Fig. 2)[47]. An interdependent relationship among genetic engineered microorganisms and modified plants can improve the effectiveness of bioremediation on contaminated sites [47].

A modified bacterium, Rhodopseudomonas capsulate, has been used in advanced swine waste treatment plants in country like Japan and Republic of Korea [48]. The concentration of short chain fatty acids are one of the main sources of the bad odor of swine wastes, decreased dramatically after treatment. The residue after treatment can be used as a safe organic fertilizer [49].

Pseudomonas fluorescens strain designated HK44, was one of genetically modified bacteria released into a contained soil environment for remediation of contaminant. Parental strain from which strain HK44 (Pseudomonas fluorescens) derivative was isolated from an industrial gas plant facility highly polluted with polyaromatic hydrocarbons [50; 51]. The pUTK21, naphthalene catabolic plasmid which was hosted into this strain to produce modified form of P. fluorescens HK44 [50]. Additionally, strain HK44 comprises a transposon based bioluminescence producing lux gene fused within a promoter for the naphthalene catabolic genes. Therefore, introduction of strain HK44 to naphthalene marks in improved catabolic expression of gene, degradation of naphthalene, and a coincident bioluminescent response [52].

The strain HK44 serves as a reporter for naphthalene bioavailability and biodegradation and, through bioluminescence signaling, can be used as an online tool for in situ monitoring of bioremediation processes [53; 54].

One of the toxic heavy metal which can be released into the environment is Mercury. Genetically modified Escherichia coli strain JM109 has capability to degrade mercury from contaminated water, soil or sediment. Genetically engineered bacteria holding the MerA gene can remove mercury from a contaminated site [52; 55]. Transgenic bacteria expressing

metallothioneins and polyphosphate kinase can promote effective mercury bioremediation [47].

In another study genetically engineered Deinococcus radiodurans and Pseudomonas putidia are capable of degrading organic pollutants in contaminated sites. The use of organophosphates in agriculture, as pesticides, has been shown to cause serious environmental pollution [47]. Genetically engineered bacteria are capable of metabolizing chlorinated organic compounds such as lindane and trichloroethylene [56].

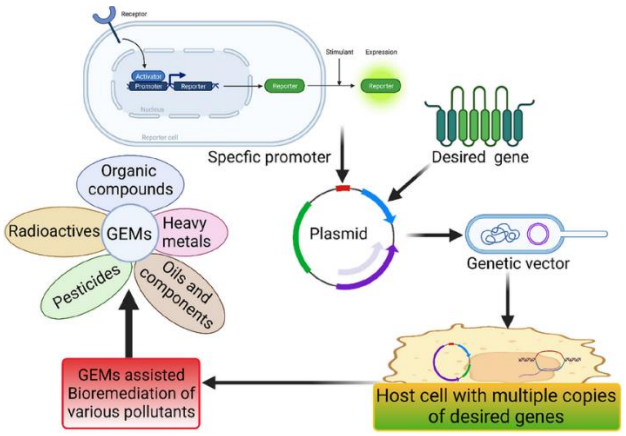

Fig. 1 Genetic engineering of microorganisms for bioremediation

VI. Importance of Genetically Engineered Microbes in Food Industry

A. *Industrial food enzyme production.*

The use of food enzymes by the industrial food industry is continuously increasing. Food enzymes are typically gained by microbial fermentation [4][107]. Optimization of fermentation process made changes in nutritional and biochemical quality of foods for which both wild-type and genetically modified strains are used to increase food enzyme production [57].

Recombinant enzymes are used to advance and increase enzyme's features, like its action, temperature optimum, and pH stability [58]. Enzymes such as amylase, maltase, proteinase, pectinase, mannase, catalase, invertase, cellulose are generally produced from fermenting microorganisms in various food industry [59]. Most genetically modified enzymes are expressed in well-established standard microbial model systems such as E. coli and P. pastoris [57].

Genetically altered strain of R. oryzae alpha amylase attained by means of site saturation mutagenesis had a higher optimal temperature and lower optimum pH than the wildtype enzyme, properties better suited for use in high maltose syrup production [60]. A mutant Aspergillus aculeatus b-glucosidase obtained using site-saturation mutagenesis had improved hydrolytic efficiency especially to cellobiose, and was used to accelerate the saccharification of alkaline-pretreated bagasse [61].

The modification of Thermotoga maritima with their substrate specificity to obtain glucosidases after genetic modification was enhanced for quercetin glucosides, suggesting its usage in producing aglycones that have higher pharmaceutical activity compared with the substrate [57]. Modified strain of Aspergillus and Bacillus species can produce food proteases this indicates that engineered metalloproteases obtained through site saturation mutagenesis of His224 had improved affinity for substrate, making the synthesis of Z-aspartame more cost-effective [62].

B. *Genetically Modified Lactic Acid Bacteria*

Lactic acid bacteria are Gram-positive bacteria with a heterogeneous group that are comprised of the genera Lactobacillus, Oenococcus, Lactococcus, Streptococcus, Enterococcus, Leuconostoc, Carnobacterium, Pediococcus, Tetragenococcus, Vagococcus and Weissella Genetic modifications in Lactic acid bacteria can be attained through plasmid encoded expression systems or chromosomal modifications [63].

Genetic alteration of lactic acid bacteria can produce in improved strains with a wide range of potential applications including for therapies, food industry and metabolite production. Live lactic acid bacteria have been developed as biotherapeutics, particularly for the treatment of gastrointestinal disorders [64]. Complete gene clusters, encoding exopolysaccharide producing enzymes have been transformed from one lactic acid bacteria strain to another one. Newly produced strains could stimulus viscosity and texture of the fermented product of our desire item for consumption [65].

The gene of S. thermophiles, the phosphoglucomutase was inactivated resulting in improved exopolysaccharide production enhancing the viscosity of the fermented food product. Engineering of exopolysaccharide production in L. lactis was also achieved by using a self-cloning strategy [66]. Genetic engineering using genes from nonrelated microorganisms could also be used to produce high added value products, such as l-alanine. By introduction of a Bacillus subtilis alanine dehydrogenase gene into a L. lactis strain deficient in lactate production, pyruvate conversion was pushed in the direction of alanine [67].

C. *Probiotics*

Probiotics were live microorganisms which administered in adequate amounts confer a health benefit on the host. Most probiotic products include lactic acid bacteria that are known as harmless for humans and animal [68]. Researchers have tried to fill the breach in the probiotic activity spectrum by improving lactic acid bacteria strains by means of genetic modification [69]. These genetically engineered probiotic strains can be used in healthcare especially for the prevention and treatment of digestive diseases and food-borne sicknesses [70]. Research on the interfaces between gut microbes, pathogenic strains and the immune system has led to the identification of key regulatory molecules that are involved in the development of different digestive diseases [71].

Most probiotics are from the genus Lactobacillus or Bifidobacterium likely to change and diversify. Similarly, the development of new therapeutic strategies, such as the development of phagebiotics, psychobiotics, and genetically modified pharmabiotics, is poised to become a therapeutic reality [72].

Through genetic engineering it has become promising to enhance the expression of these bioactive compounds using

probiotic strains. Some of the targets for the genetic modification of probiotic strains have included host gut immunomodulation, antimicrobial compound release, vitamin synthesis, short-chain fatty acids and polysaccharides with prebiotic effects, adhesins, and digestive enzymes [73].

Intestinal disorder initiated by Inflammatory bowel disease caused by persistent inflammation of gastrointestinal tract due to diminished immune response to the gut microbiota. The maintenance of intestinal homeostasis needs a balance between gut microbiota and immune cells deregulation of these immunological interactions leads to inflammation [56]. Several studies have produced genetically engineered probiotic strains that produce anti-inflammatory effects. Genetically modified Lactococcus lactis LL-Thy12 strain substituted the thymidylate synthase gene with a synthetic sequence encoding mature human interleukin-10. This food-grade strain was able to secrete anti-inflammatory cytokines (IL-10) and preclinical studies have revealed it be effective in the treatment of colon inflammation [69].

D. Genetically Modified Microorganism as Biosensor

Microbial biosensor are dense, portable, handy, cost effective and simple to use making them in situ monitoring of environmental toxic waste. An exciting application of genetically engineered microorganism is their use as sensor for biologically relevant concentrations of agrochemical, petroleum products, heavy metal and toxin in environment sample [74]. Furthermost microorganism used as biosensor are modified with reporter genes to promoter that induce its expression depending on nature and concentration of the compound of interest. Genetically engineered microbe's biosensor kits and high throughput cell arrays on chips, optic fibers or other platforms for onsite and online monitoring of environmental pollution [75].

The selection of the promoter determines the specifically to certain compounds [74]. Modified autotrophic E. coli strain for the detection and quantification of mevalonate, an in-between in the biosynthesis of isoprenoids, a large class of industrially significant secondary metabolites that comprises flavor, perfume, anti-oxidants, steroids, and the anti-malarial drug artemisinin [76].

Production of isoprenoid consists mevalonate as it key precursor, enhancing the level of mevalonate is significant in increasing recombinant strains for enhanced isoprenoid production [51]. Genetically engineered microorganisms based on combining of the lux, gfp, lacZ gene reported to an inducible gene promoter have been widely applied to assay toxicity and bioavailability [76].

E. Genetically Engineered Microorganisms as Biopesticides

Biopesticides are all ingredients or substance derived from natural materials, including plants, animals and micro-organisms, that exhibit pesticidal activity. Such biological regulator agents are gradually targeted for genetic improvement due to a growing recognition of their potential advantage to present agriculture [77].

Biological control represents a substitute to chemical pesticides which have been imperiled to much blame due to their contrary impacts on the environment and human health. Consequently, there is a strong prerequisite to advance safer and environmentally amenable pest control using existing organisms in their natural habitat [78]. Several such organisms' biological control agents, are available that offer protection against a wide range of plant pests and pathogenic microbial agents without damaging the ecosystem [79].

The foreign genes used for transforming biological control agents can be integrated into the host genome or a plasmid. To express a heterologous gene in fungi or bacteria, the regulatory region of this gene must be modulated in its promoter and terminator regions in order to optimize the expression of the inserted gene in the new host [80]. Adding of particular genes that are recognized to confer biocontrol activity may improve biocontrol capability of organisms that do not naturally own these genes [79]. Several significant rhizobacteria including Sinorhizobium meliloti and P. putidrii, both of which are outstanding root colonizers, lack the capacity to produce chitinases. Chitinases are enzymes that terminate chitin, a major constituent of fungi cells. Introducing genes encoding chitinases into their genome have assisted them to provide protection against plant pathogenic fungi [81].

Sinorhizobium is a symbiotic bacterium which stimulates formation of root nodules in legumes involved in fixing atmospheric nitrogen. Many Pseudomonas species in the rhizosphere environment produce siderophores which chelate iron ions, thereby increasing iron uptake by plants [82]. The genetically modified commercial strain (RmBPC-2) of S. meliloti has added genes that regulate the nitrogenase enzyme involved in nitrogen fixation [83].

Several extracellular enzymes, including chitinases, glucanases, lipases and proteases, are produced by the Trichoderma species, which has been improved further with the transfer of chitinase genes, notably from Serratia marcescens and other entomopathogenic bacteria that produce pest specific toxins are environmentally friendly alternative to chemical insecticides [84]. Genetic engineering has great potential for the development of newly engineered entomopathogens [85].

The Agrobacterium radiobacter strain k84 protects plants against crown galls caused by A. tumefaciens strains carrying Ti-plasmids of the nopaline type. Protection conferred by A. radiobacter strain k84 is due to agrocin 84, an A nucleotide derivative. A. radiobacter has an additional negative effect on soil pathogens by being a very effective rhizosphere colonizer [86]. Even though A. radiobacter strain k84 has been widely used commercially for a long time, there was concern about its long-term efficacy as a biocontrol agent. This is because the gene encoding agrocin is carried on a transmissible plasmid, which can be transferred by conjugation to A. tumefaciens. In the event of agrocin-encoding plasmid transfer, recipient A. tumefaciens strains would no longer be subjected to biocontrol by A. radiobacter strain k84 [87]. The ensuing genetically engineered strain, known as A. radiobacter strain K1026, is a transgenic organism approved for use as a pesticide [88].

Bacillus thuringiensis has been used as a biopesticide for several years. The insecticidal action of B. thuringiensis is based on the creation of crystalline protein inclusions during sporulation. The crystal proteins are encoded by different cry genes and are also known as delta-endotoxins [89]. The protein

crystals are highly toxic to a variety of important agricultural insect pests; when the proteins are taken up by susceptible insect larvae they induce lysis of gut cells, resulting in death of the larvae by starvation and sepsis [90].

The transgenic P. flourescens strains are killed and used as a more stable and persistent biopesticide compared to the B. thuringiensis sprays. Moreover, cry genes are widely used to create transgenic plants that directly express the toxin and are thus protected from susceptible insect pests. Baculoviruses are also being manipulated to be effective biopesticides against insect pests such as corn borer, potato beetle and aphids [53].

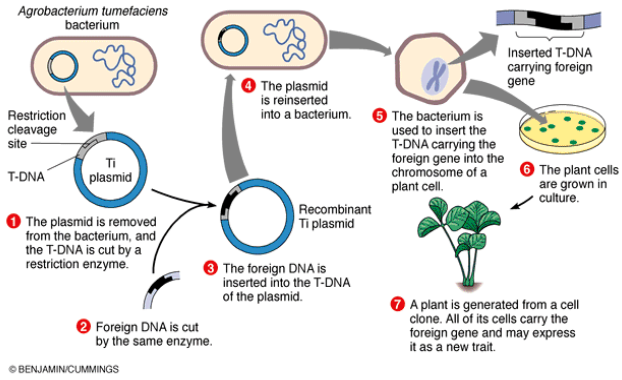

Fig. 2 Steps by using Agrobacterium for genetic engineering

VII. Risks of Genetically Engineered Microorganisms

The introduction of a gene into different cells can result in different result, and the overall pattern of gene expression can be altered by the introduction of single gene. The insertion of single can impact the entire genome of the host resulting in unintended side effects [91; 92]. Furthermore, work on Escherichia coli and Pseudomonas has shown that the non-homogeneous texture of natural niches selects distinct populations that quickly diverge genetically from the initial inoculum. One risk associated with genetic engineering is that it is based on the idea that each trait of an organism is encoded in a single, specific gene, and that the transfer of that specific gene will also cause the transfer of the sought-after attribute [93].

A. Impact on the health of people

There have been concerns that eating genetically modified foods can contribute to the development of cancer by rising levels of possibilities of carcinogenic ingredient in the body. There is a risk that the microbes might pass to the human genes they carry to other bacteria in the body, with unknown consequences [24]. Impending adverse effects include enhanced pathogenicity, emergence of a novel disease, increased disease burden if the recipient organism is a pathogenic microorganism or virus [94]. Most of these models are related with the growing and ingesting of genetically engineered or modified crops. Different impacts could be allied with genetically modified animals and, like the risks related with plants, would depend largely on the new traits introduced into the organism [95; 96].

B. Ecological impacts

Modern agriculture with genetically engineered microbes affords the potential for maintainable feeding of the worlds growing population. The effects of deviations in a single species might spread beyond to the ecological unit [97]. Single impacts are always combined by the risk of ecosystem damage and destruction. In particular, studies linking genetically modified traits on ecosystem processes at longer time scales are rare, but needed for assessing trait effect, especially in an evolutionary context [98]. The impact of introduction of genetically modified microorganism on the soil ecosystem can shift of certain species of microorganism and can alter population structure and function by disturbing key ecological process [99].

C. Allergens in the food supply

Genetic engineering of microorganism may have resulted in a new protein or a known allergen was introduced. The problematic is unique to genetic engineering because it alone can transfer proteins across species boundaries into completely unrelated organisms [100]. Genetic engineering routinely moves proteins into the food supply from organisms that have never been consumed as foods. Some of those proteins could be food allergens, since virtually all known food allergens are proteins [101]. It is challenging to forecast whether a protein expressed in a new organism might cause allergies. Recent research substantiates concerns about genetic engineering rendering previously safe foods allergenic [72]. Newly expressed proteins from genetically modified crop those contains genes from modified microorganism have capability to sensitive immune system to respond abnormally to alike proteins due to cross reactivity with alternative allergen [101].

D. Antibiotic resistance

Human or animals exposed to probiotic strains holding antibiotic resistance gene that might be transferred to the commensal microbiota in vivo are at a risk mostly horizontal gene transfer can able to produce this effect. Genetic engineering often uses genes for antibiotic resistance [102]. Most genetically modified plant foods transfer fully effective antibiotic resistance genes. The occurrence of antibiotic resistance genes in foodstuffs may have adverse effects. Consumption of these foods could decrease the efficacy of antibiotics to combat disease when these antibiotics are taken with meals. Antibiotic resistance genes produce enzymes that can degrade antibiotics. The resistance genes could be transferred to human or animal pathogens, making them impervious to antibiotics [103]. Whenever were to occur, it could aggravate the already serious health problem of antibiotic-resistant disease organisms. While unmediated transmissions of genetic material from plants to bacteria are highly unlikely, any possibility that they may occur requires careful scrutiny in light of the seriousness of antibiotic resistance [4].

E. Concentration of toxic metals

More or less the new genes being introduced to crops with genetically engineered microbes can eliminate heavy metals like mercury from the soil and quintessence them in the tissues of plant. The aim of making such crops is to make possible the use of public sludge as biofertilizer. Sludge encompasses valuable plant nutrients, nevertheless cannot be used as fertilizer because it is contaminated with toxic heavy metals [77]. Whirling on the genes in merely some portions of the plants needs the use of genetic switches that turn on only in exact tissues, like leaves. There are also environmental risks associated with the handling

and disposal of the metal-contaminated parts of plants after harvesting [104].

Modern technology is not free from risks, the full set of threats related with genetic engineering have almost surely not been recognized. The capability to conceive what might go wrong with a technology is inadequate by the currently incomplete understanding of physiology, genetics, and nutrition [4, 93, 105]

VIII. Conclusion

Genetic engineering microorganism is a recent technology that artificial manipulation, modification and recombining DNA or other nucleic acid to modify characteristic of microbes for the benefit of individual. Genetic engineering of microorganisms in many respects much easier compared to plants and animals. Microorganisms are advantages due to their growth rate. It has a number of useful application in bioremedy, biopesticide, biosensor, food enzyme production and probiotic. (CRISPR)/CRISPR-associated protein 9 (Cas9) represented a major breakthrough in industrial biotechnology mainly used for selection of recombinant strains. Genetic modification of lactic acid bacteria can result in upgraded strains with a wide spectrum of possible applications, including for therapies, the food industry and metabolite production. However, the application of genetically engineered microorganisms, the risk of horizontal gene transfer and consequent distribution of antimicrobial resistance. One of the criticisms of genetically engineered microorganisms' tools is that it might not be safe. That does not mean that specific transgenic solutions are risk free. More advanced and better technological advancement techniques should be developed and applied in the genetic engineering of microbes to minimize risks. Detail studies are required which assess the risks of GMO (Genetically Modified Organism) crops those integrated with genetically engineered microorganism special emphasis should be given.

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