Chapter 1
Genetically Modified Organisms (GMOs) and Their Potential in Environmental Management: Constraints, Prospects, and Challenges

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Abstract Increasing environmental contamination with highly toxic chemicals is warning us to find sustainable technologies to protect the environment and human health, which is a key challenge of the current scenario. A variety of physicochemical technologies are currently being applied presently to decontaminate the environment to safeguard the environment and human health. However, these technologies are costly and chemical-consuming, thus causing secondary pollution and, hence, are not environmental-friendly. As an alternative approach, bioremediation technologies using microbes and plants and their enzymes are currently viewed as eco-friendly and most sustainable technologies due to their self-sustainable and low-cost nature. But sometimes bioremediation technologies are get limited by low degradability/accumulability of microbes and plants, respectively. To overcome these limitations, genetic engineering approaches are highly decisive to design the transgenic microbes and plants for the enhanced biodegradation and biodetoxification of environmental pollutants for sustainable development. Genetically modified organisms (GMOs) offer great potential for environmental remediation, and hence, in this chapter, we focused on the applications of GMOs in the environmental management with risks involved, constraints, and challenges faced by researchers in the release of GMOs for field applications.

Keywords Environmental pollutants · Genetically modified organisms · Environmental remediation · Transgene · Genetic engineering

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1 Introduction

In the last few decades, due to industrialization, increase in population, and daily life requirements, harmful chemicals have been released into the earth's air, soil, and water (Goutam et al. 2018; Gautam et al. 2017; Bharagava et al. 2017a, b; Saxena et al. 2016; Olugbenga 2017). Excessive mining, agriculture waste, and burning of fossil fuels consequently release enormous amounts of toxic heavy metals like Hg, Pb, U, Cd, Zn, Cr, Ni, Co, and Cu and metalloids (As) into the environment which create mutagenic and carcinogenic effect (Wernick and Themelis 1998; Wijnhoven et al. 2007). Several chemical industries use and produce wide varieties of hazardous compounds like benzene, toluene, polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), dioxins, nitro-aromatics, dyes, polymers, pesticides, explosives, chlorinated organic, and pharmaceuticals (Meagher 2000; Pilon-Smits 2005).

Moreover, many of these substances are non-biodegradable and persistent in nature that stay long in our natural environment. Many of these substances are toxic and cause a harmful effect on human health and damage the ecological balance. However, there is an urgent need to remove these compounds for environmental and public health safety. The remediation and restoration of sites contaminated with highly toxic and hazardous pollutants requires eco-friendly and effective approach for environmental sustainability and to safeguard the public health. Microbial bioremediation is a waste management technology which uses microorganisms like bacteria, algae, and fungi to degrade and transform hazardous compounds of soil and water, while phytoremediation is cost-effective and environmental-friendly technology that has a potential application to efficiently degrade and transform organic and inorganic pollutants (Kishor et al. 2018; Saxena and Bharagava 2016; Bharagava et al. 2017c, 2019; Meagher 2000).

Eventually, naturally occurring microorganisms are incapable of degrading all toxic compounds, especially xenobiotic. To overcome this, serious efforts have been done to create genetically engineered microorganisms (GEMs) to enhance bioremediation approaches besides degrading xenobiotic (Sayler and Ripp 2000). Thus, biotechnology is a most important technique that has been applied in different areas especially in remediation to neutralize various unfit complex environmental pollutants into nontoxic or simple form and to completely remediate organic wastes (Iwamoto and Nasu 2001). Recombinant DNA technology has been studied intensively to improve the biodegradation of hazardous pollutants in lab conditions (Dua et al. 2002). In the late 1970s and early 1980s, the cloning and characterization of bacterial genes that code for catabolic enzymes for the biodegradation of recalcitrant pollutants has started. The organism whose genetic material, i.e., DNA, has been modified/altered in such a way so as to get the required traits is often called as genetically modified organism (Shukla et al. 2010; Liu et al. 2011). This technology is often called "gene technology," or "recombinant DNA technology" (RDT), or "genetic engineering," and the resulting organism is said to be "genetically modified," "genetically engineered," or "transgenic."

In addition, the leakage and industrial discharge of petrol and their associated chemicals like polycyclic aromatic hydrocarbons (PAH) pose a highly negative impact on aquatic and terrestrial ecosystems. Genetically modified organisms (GMOs) have a capability to clean up and remove industrial waste and pollutants from the environment as well as reduce toxicity of elements (Liu et al. 2011).

Genetic engineering is currently popular among researchers worldwide to develop new microbes with required traits as compared to its wild type for the degradation and detoxification of a wide range of xenobiotic compounds (Kumar et al. 2013).

In 1970, the first GMOs called "superbug" were developed by genetic engineering through plasmid transfer that have ability to degrade a variety of petroleum chemicals such as xylene, camphor, hexane, naphthalene, and toluene. GMOs are capable for enhanced degradation and removal of a wide range of xenobiotic and also have potential application for bioremediation of environmental pollutants (Kulshreshtha 2013). Designing of GMOs primarily depend on the knowledge of genetic basis of interaction between microbes and xenobiotic compounds, structure of operon, molecular biology, biochemistry, and ecology (ref). Thus, GMOs can be potential molecular tools to degrade and detoxify the environmental pollutants in contaminated matrix to safeguard the environment and public health. Therefore, this chapter has mainly focused on the role of GMOs in the bioremediation of organic and inorganic pollutants, constraints in utilizing them in bioremediation, and limitations in field applications.

2 Genetically Modified Organisms

Designing of suitable genetically modified organisms (GMOs) for enhanced bioremediation of environmental pollutants from contaminated matrix requires creation of new routes for metabolism, intensifying a range of existing degradation pathways, avoiding substrate misrouting into unproductive routes or to toxic metabolite generation, improving the substrate flux through degradation pathways to avoid the accumulation of toxic intermediates, enhanced stability of catabolic potential, enhanced bioavailability of hydrophobic pollutants, and enhanced catabolic potential of microbes (Timmis and Pieper 1999; Pieper and Reineke 2000; Furukawa 2003).

Although an organism produced from genetic engineering techniques allows the transfer of specific functional genes into a particular organism genome (Tozzini 2000). A US definition of GMO, "genetically modified organisms," refers to microorganism, plants, and animals containing distinctive genes transferred from other species to produce unique characteristics to completely clean up and mineralize hazardous waste material. Many bacterial strains such as *Bacillus idriensis*, *Ralstonia eutropha*, *Sphingomonas desiccabilis*, *Pseudomonas putida*, *Escherichia coli*, *Mycobacterium marinum*, etc. have been used to design genetically engineered microbes with insertion of a functional gene into other species which capable for the bioremediation of heavy metals and non-biodegradable compounds of contaminated environment (Valls et al. 2000; Ackerley et al. 2004; Kube et al. 2005; Parnell et al. 2006; Schue et al. 2009; Liu et al. 2011).

Moreover, the genetic engineering of plants also performed to enhance the accumulation and tolerance capacity as well as detoxification potential for heavy metal pollutants and to increase the biomass and growth of plants in metal contaminated sites (Hassani 2014). Metallothioneins (MTs) are the unique cysteine-rich peptides that are relevant to higher metal-binding capacity in hyperaccumulating plants and have been cloned to develop the genetically engineered plants for phytoremediation of organic and inorganic pollutants. Tobacco plant was the first genetically engineered plant for the phytoremediation of explosives and halogenated organic pollutants (Doty et al. 2000). Many reports have been published on the genetic engineering of plants and their role in the phytoremediation of contaminated soil and water environment (Cherian and Oliveira 2005; Pilon-Smits 2005; Eapen et al. 2007; Doty 2008; Macek et al. 2008; James and Strand 2009; Kawahigashi 2009; Van 2009). Recently, James and Strand (2009) reported the dehalogenation of tetrachloroethylene (PCE) by hybrid poplar trees under controlled field conditions. Genetically modified organisms can be also used as biosensors for related mixures of agrochemicals, petroleum products, metals, and toxins that are found in the environment, but cannot be directly in soil or water (Ozcan et al. 2011).

3 Environmental Bioremediation Technologies

Environmental bioremediation technologies broadly can be classified into two major categories: bioremediation and phytoremediation.

3.1 Bioremediation

Bioremediation is the eco-friendly technique wherein biological agents (microbes and plants or their enzymes) are used to degrade and detoxify the organic and inorganic pollutants to safeguard the environment and public health in low-cost and efficient manner (Azubuike et al. 2016; Bharagava et al. 2018; Kishor et al. 2018). A range of bioremediation techniques have been developed by researchers to date; but due to diverse characteristics of pollutants and merits and demerits, no single bioremediation technique can provide full-scale solution to contaminated environment (Verma and Jaiswal 2016). Microbes that are involved in the degradation and detoxification of organic and inorganic pollutants are *Mycobacterium*, *Acinetobacter*, *Flavobacterium*, *Actinobacteria*, *Alcaligenes*, *Beijerinckia*, *Arthrobacter*, *Methylosinus*, *Bacillus*, *Micrococcus*, *Serratia*, *Nitrosomonas*, *Rhizoctonia*, *Pseudomonas*, *Nocardia*, *Phanerochaete*, *Penicillium*, *Xanthobacter*, and *Trametes*. Bioremediation involves three main processes: biotransformation (conversion of organic and inorganic pollutants into less or nonhazardous molecules), biodegradation (breakdown of complex organic pollutants into simple and smaller unit molecules), and mineralization (complete biodegradation of organic matter into inorganic constituents such as CO_2 or H_2O) (Saxena and Bharagava 2017; Saxena and Bharagava 2015; Pilon-Smits 2005).

On the basis of application potential, bioremediation can be applied as ex situ and in situ. In situ bioremediation technologies involve treatment of pollutants at the site of pollution, do not require any excavation means, do not pose any disturbance to soil environment, and require continuous oxygen supply for proper aeration to support the microbial growth for degradation of contaminants (Vidali 2001). In situ bioremediation technologies are cost-effective as these uses microbes for pollutant removal from contaminated matrix and for the degradation and detoxification of polyaromatic hydrocarbons, azo dyes, chlorinated solvents, and heavy metals (Kumar et al. 2011; Folch et al. 2013; Kim et al. 2014; Frascari et al. 2015; Roy et al. 2015). In situ bioremediation technologies are biosparging, bioventing, and phytoremediation.

Ex situ bioremediation technologies involve the treatment of pollutants at any place other than the site of pollution and require excavation of contaminated soil or pumping of groundwater to enhance the microbial degradation process. These remediation approaches are costly, and their applicability depends on the pollutants type, pollution strength and depth, and geographic conditions of contaminated sites (Philp and Atlas 2005). These approaches are classified into two methods: solid phase system (including land treatment and soil piles) and slurry phase systems (including solid liquid suspensions in bioreactors) (Kumar et al. 2013).

3.2 Phytoremediation

Phytoremediation is an eco-friendly phytotechnology that involves the use of plants/trees for the treatment and restoration of contaminated sites/wastewaters/groundwater (Saxena et al. 2019; Chandra et al. 2015). By using green plants, the pollutants such as metals, pesticides, herbicides, explosives, oil, solvents, and their derivatives can be removed and cleaned up from polluted and contaminated soil, streams, and groundwater (Meagher 2000; Pilon-Smits 2005). Phytoremediation technologies may be inexpensive and harmless process than traditional ones and offer easy plant control and re-use of valuable metals. Exudates released by roots in the rhizosphere of plants also support the growth of soil beneficial microbes that participate in the degradation and detoxification of pollutants (rhizoremediation), and chelating agents help to convert non-available elements into bioavailable forms for plant uptake for growth (Suresh and Ravishankar 2004; Abhilash et al. 2009).

The genetically engineered plants have been developed through transgenic engineering to degrade and detoxify the organic and inorganic pollutants (Zhu et al. 1999; Abhilash et al. 2009). The increased accumulation of pollutants (in case of heavy metals) facilitates their removal from contaminated matrix and, thus, prevents their migration to other environments where these can create pollution and health hazards to living beings. However, phytoremediation has some disadvantages such as limitation to the surface area and depth occupied by the roots, slow plant growth, low biomass production, and contamination possibility of food chain by accumulated contaminants (Macek et al. 2008). Phytoremediation covers several different strategies such as phytoextraction, rhizofiltration, phytostabilization, phytovolatilization, etc. (Eapen and D'Souza 2005; Cherian and Oliveira 2005; Doty 2008; Macek et al. 2008).

4 Genetically Engineered Bacteria in Bioremediation of Heavy Metals and Organic Pollutants

Water and soil are essential components of all living things on earth. But unfortunately these are contaminated by geogenic and anthropogenic activities like mining, volcanic eruption, heavy rainfall, industrializations, urbanization, and agriculture waste, which are liable for the pollution of our natural environment and toxicity in the living beings. Therefore, it is urgent need to adequately treat the contaminated water and soil to protect the environment and public health. There are several reports available on the bioremediation of heavy metals and organic pollutants by different microorganisms (Strong et al. 2000; Barac et al. 2004). Genetically engineered bacteria reported in the degradation and detoxification of organic and inorganic pollutants are listed in Table 1.1.

A variety of potential strains of bacteria such as *Bacillus idriensis*, *Ralstonia eutropha*, *Sphingomonas desiccabilis*, *Pseudomonas putida*, *Escherichia coli*, *Mycobacterium marinum*, etc. have been genetically engineered for the enhanced bioremediation of toxic heavy metals in the contaminated matrix (Valls et al. 2000; Deng et al. 2003; Ackerley et al. 2004; Deng et al. 2005; Kube et al. 2005; Parnell et al. 2006; Singh et al. 2008; Schue et al. 2009; Liu et al. 2011). Bioremediation of Hg is mainly facilitated by transgene that confers arsenic resistance to microbes such as mer operon genes (Jan et al. 2009), mercuric ion transporter gene merC in *Acidithiobacillus ferrooxidans* (Sasaki et al. 2005), and mercuric ion transporter gene merH in *Mycobacterium marinum* (Schue et al. 2009). The genetically engineered radiation-resistant bacterium, *Deinococcus radiodurans*, also showed a great potential for the bioremediation of radioactive waste containing mercury ion (Brim et al. 2000). The genetically engineered mercury-resistant bacterium, *Escherichia coli* (merT-merP and MT genes), also showed a huge potential for the removal of Hg^{2+} from electrolytic wastewater (Deng and Wilson 2001). It has been also reported that the accumulation of Cd^{2+} was enhanced into *Mesorhizobium huakuii* when transformed with a gene that code for phytochelatins from *Arabidopsis thaliana* (Sriprang et al. 2003).

Kang et al. (2007) reported that the recombinant *E. coli* can accumulate Cd up to 25-fold more than control strain. Wu et al. (2006, 2010) studied the alleviation of Cd toxicity using a metal-binding peptide (EC20) expressing rhizobacterium,

Table 1.1 Genetically modified bacteria (GMBs) for enhanced bioremediation of organic and inorganic pollutants

GMBs	Introduced gene(s)	Pollutants	References
Pseudomonas putida PaW340(pDH5)	pDH5 plasmid	4-chlorobenzoic acid	Massa et al. (2009)
Escherichia coli JM109 (pGEX-AZR)	Azoreductase gene	Azo dyes, C.I. Direct Blue 71	Jin et al. (2009)
Pseudomonas putida pnrA	Nitroreductase	TNT	Van Dillewijn et al. (2008)
Pseudomonas putida PaW85	pWW0 plasmid	Petroleum	Jussila et al. (2007)
Rhodococcus rhodochrous XplA, XplB	Cytochrome P450 monooxygenase	RDX	Jackson et al. (2007)
Enterobacter cloacae NfsI	Nitroreductase	TNT	Hannink et al. (2007)
E. coli NfsA	Nitroreductase	TNT	Kurumata et al. (2005)
B. subtilis BR151 (pTOO24)	Luminescent Cd sensors	Cd (Naturally polluted soils)	Ivask et al. (2011)
Sphingomonas desiccabilis and *Bacillus Idriensis* strains	Over expression of arsM gene	As (Laboratory conditions)	Liu et al. (2011)
Methylococcus capsulatus (Bath)	CrR genes for Cr (VI) reductase activity	Cr (VI) (Cell-associated Cr removal in laboratory conditions)	Hasin et al. (2010)
Pseudomonas strain K-62	MerE protein encoded by transposon Tn21 (broad Hg transporter)	Hg (Across the bacterial membrane)	Kiyono et al. (2009)
Bacillus megaterium strain MB1	mercuric ion binding protein (MerP)	Hg	Hsieh et al. (2009)

Pseudomonas putida 06909. Patel et al. (2010) studied that a recombinant bacterial strain, *Caulobacter crescentus* JS4022/p723-6H, expressing RsaA-6His fusion protein can remove up to 99.9% of the Cd as compared to control bacterium which can remove up to 37% of Cd. Arsenic removal from contaminated matrix has been also studied using recombinant microbes by several workers (Valls and de Lorenzo 2002; Qin et al. 2006; Yuan et al. 2008). A recombinant bacterium, *E. coli* (containing arsM gene from *Rhodopseudomonas palustris*), can transform highly toxic inorganic As into less toxic volatile trimethylarsine (Qin et al. 2006; Yuan et al. 2008). Further, a recombinant bacterium, *E. coli* SE5000 strain (containing nixA gene), can also accumulate Ni^{2+} from aqueous solution (Fulkerson et al. 1998).

Further, it has been reported that the Ni resistance was enhanced in the recombinant *E. coli* when introduced with the serine acetyltransferase gene from Ni hyperaccumulating plant, *Thlaspi goesingense* (Freeman et al. 2005). Recently, Hasin et al. (2010) have characterized a methanotrophic bacterium, *Methylococcus capsulatus*, which can successfully bioremediate Cr^{6+} in a wide range of concentrations (1.4–1000 mgL^{-1} of Cr^{6+}). However, a recombinant Cd-resistant rhizosphere bacterial strain, *Pseudomonas putida* 06909, could detoxify Cd due to its ability to produce metal-binding peptide (MBP)-EC20 that has high affinity for Cd (Lee et al. 2001).

In 1970, the first GEMs called "superbug" were constructed to degrade oil by the transfer of plasmids which could utilize a number of toxic organic chemicals like octane, hexane, xylene, toluene, camphor, and naphthalene. Microorganisms that are well adapted to survive in the soil environment may not be able to survive in aquatic environment and hence cannot be used successfully. Therefore, aquatic microbes can be used to develop GEBs for bioremediation of aquatic sources. The use of such organisms would avoid the supplementation of nutrients to the inoculated environment, thereby reducing the costs incurred and maintenance required (Kulshreshtha 2013). Scientists have developed *Anabaena* sp. and *Nostoc ellipsosporum* by the insertion of linA (from *P. paucimobilis*) and fcbABC (from *Arthrobacter globiformis*), respectively. The gene linA responsible for the biodegradation of lindane (γ-hexachlorocyclohexane), and fcbABC confers the ability to biodegrade halobenzoates and can be used to remediate these pollutants from water sources. GEBs have been developed by hybrid gene clusters which alter their enzymatic activity and substrate specificities (Kulshreshtha 2013). These gene clusters encode the enzyme possessing improved transforming capability. *E. coli* strain is genetically modified to express a hybrid gene cluster for the degradation of trichloroethylene (TCE) (Kulshreshtha 2013). GEMs possess chemical sensors that allow the monitoring of contaminant bioavailability rather than just contaminant presence (Kumar et al. 2013). Bioluminescence-producing GEMs also help us to understand the spread of microbes in the polluted area and end point of the bioremediation (Kulshreshtha 2013).

The genetically engineered *Pseudomonas* strains were the first microbe developed by Indian-born American scientist Dr. Anand Mohan Chakrabarty, with high catalytic potential to the subject of intellectual property right [US Patent #425944], which could degrade a variety of petroleum hydrocarbons such as naphthalene, camphor, xylene, octane, and salicylate. Following the seminal work of Chakrabarty and his colleagues on the degradation of petroleum and chloroaromatic compounds (Harvey et al. 1990; Haugland et al. 1990), the possibilities of using genetic engineering technique in biodegradation of organic pollutants had received a breakthrough with many papers published by the Timmis Laboratory in the mid- and late 1980s (Ramos et al. 1987; Rojo et al. 1987). Thus, genetic engineering techniques have been proved to be an efficient molecular approach for the microbial bioremediation of pollutants.

5 Genetically Engineered Plants in Phytoremediation of Heavy Metals and Organic Pollutants

Phytoremediation is the engineered use of green plants/trees with associated microbiota for the degradation and detoxification of organic and inorganic pollutants from the contaminated matrix (soil/water) to safeguard the environment and public health. Genetically engineered plants were first developed for the phytoremediation of heavy metals (Misra and Gedamu 1989; Rugh et al. 1996). However, the tobacco plants were the first genetically engineered plants for the phytoremediation of organic pollutants (explosives and halogenated organic compounds) (Doty et al. 2000). Genetically engineered plants are developed by introducing the transgene of interest that are responsible for the metabolism of xenobiotic compounds and offer increased resistance to pollutants (Abhilash et al. 2009). Due to the increased capacity to accumulate toxic metals from contaminated matrix, plants are chiefly preferred for the phytoremediation of heavy metals-contaminated sites. After phytoremediation, the aboveground harvestable plant biomass is safely disposed of or utilized to recover the valuable metals for future use (Salt et al. 1998). Genetically engineered plants used for the phytoremediation of environmental contaminants are listed in Table 1.2.

Phytoremediation has several advantages over microbial bioremediation approaches such as high biomass of the remediating plants with less nutrient requirements, which prevent migration of pollutants from one place to another and greater acceptance among public (Alkorta et al. 2004). The best known metal hyperaccumulating plant is alpine pennycress, *Thlaspi caerulescens*, which hyperaccumulates Zn^{2+}, Cd^{2+}, and Ni^{2+} from contaminated matrix (Milner and Kochian 2008; Baker et al. 2000). Members of Brassicaceae, *Alyssum* sp. (a serpentine-endemic shrub), *Astragalus racemosus*, *Leguminosae milkvetch*, and Indian mustard *Brassica juncea*, are known to accumulate high concentration of heavy metals from contaminated environment (Reeves and Baker 2000). Recently, Asian stonecrop, *Sedum alfredii* of Crassulaceae, has gained more attention to researchers as it hyperaccumulates Pb^{2+} and Cd^{2+} and Zn^{2+} with more than 2% of shoot weight (Yang et al. 2003; Lu et al. 2008; Deng et al. 2008).

Further, the genetically engineered, fast-growing, and high-biomass-producing metal hyperaccumulators with required genetic traits have been proved to be the suitable candidates for the phytoremediation of contaminants and include shrub tobacco *Nicotiana glaucum*, *B. juncea*, yellow poplar *Liriodendron tulipifera*, and sunflower *Helianthus annuus* (Eapen and D'Souza 2005). Several publications have reported the potential of phytoremediation to restore the polychlorophenol-contaminated soil/water (Newman and Reynolds 2004). Different plant-based remediation approaches are known including the rhizosphere biodegradation of chlorophenols inside the plant tissues (Van 2009). de Araujo et al. (2002) showed that *Agrobacterium rhizogenes*-transformed roots removed up to 90% phenolics, including phenol, 2-chlorophenol (2-CP), 2,6-dichlorophenol (2,6-DCP), and 2,4,6-TCP, from culture medium within 120 h. Sandermann (1994) studied the plant

Table 1.2 Genetically engineered plants (GEPs) for enhanced phytoremediation of organic and inorganic pollutants

Gene	Origin	Target plant	Pollutants	References
AtACR2	*A. thaliana* L.	*Nicotiana tabacum*	As	Nahar et al. (2017)
StGCS-GS	*Streptococcus thermophilus*	*Beta vulgaris* L.	Cd, Zn and Cu	Liu et al. (2015)
MerE	*E.coli* XL1-Blue	*Arabidopsis thaliana* L.	Methyl-Hg and Hg	Sone et al. (2013)
CYP2E1 and *GST*	*Homo sapiens*	*Homo sapiens* Alfalfa *(Medicago sativa)*	Hg and Trichloroethane	Zhan et al. (2013)
ScYCF1	*S. cerevisiae*	*Populus alba X P.*	Cd, Zn and Pb	Shim et al. (2013)
YCF1	*S. cerevisiae*	*Brassica juncea* L.	Cd and Pb	Bhuiyan et al. (2011)
tcu1	*Neurospora crassa*	*Nicotiana tabacum* L.	Cu and Zn	Singh et al. (2011)
tzn1	*Neurospora crassa*	*Nicotiana tabacum* L.	Cd, Fe, Ni, Cu, Mn and Pb	Dixit et al. (2010)
PsMTA1	*Pisum sativum* L.	*Populus alba* L.	Cu	Balestrazzi et al. (2009)
TnMERI1	*Bacillus megaterium*	*A. thaliana*	Hg	Hsieh et al. (2009)
GSH1	*S. cerevisiae*	*A. thaliana* L.	Cd and As	Guo et al. (2008)
GSH1 and *AsPCS1*	*S. cerevisiae* and *A. sativum*	*A. thaliana* L.	Cd and As	Guo et al. (2008)
AtPCS1	*A. thaliana* L.	*B. juncea* L	Cd and As	Gasic and Korban (2007)
CYP1A1, CYP2B6, CYP2C19	*Homo sapiens*	*Oryza sativa*	Herbicide (atrazine, metolachlor)	Kawahigashi et al. (2008)
GstI-6His	*Zea mays*	*N. tabacum*	Alachlor	Karavangeli et al. (2005)
TaPCS1	*T. aestivum*	*N. glauca*	Pb and Cd	Gisbert et al. (2003); Martinez et al. (2006)
P1A1, CYP2B6, CYP2C9, CYP2C19	*Homo sapiens*	*Solanum tuberosum*, *Oryza sativa*	Sulfonylurea and other herbicides	Inui and Ohkawa (2005)
atzA	Bacteria	*Medicago sativa*, *N. tabacum*	Atrazine	Wang et al. (2005)

metabolism of 2,4-D, including hydroxylation of the aromatic ring (*Phase I*), conjugation with *O*-manolyl-glucoside (*Phase II*), and deposition into the vacuole (*Phase III*). Burken and Schnoor (1998) also studied the degradation of [14C]-atrazine into less toxic metabolites inside hybrid poplar trees. Cytochrome P-450s have been reported to oxidize many chlorinated pesticides, including chlorotoluron, linuron, atrazine, and isoproturon (Kawahigashi et al. 2007).

Banerjee et al. (2002) reported that the transgenic hairy root cultures of *Atropa belladonna* (developed by introducing rabbit cytochrome P-450 2E1) can metabolize trichloroethane at very fast rate as compared to its wild type. Doty et al. (2007) successfully performed the transgenic engineering of poplar plants (*Populus deltoides* × *Populus alba*) overexpressing mammalian cytochrome P450 2E1 (CYP2E1) for the enhanced degradation of trichloroethane, carbon tetrachloride benzene, and chloroform.

6 Constraints, Risks, and Challenges in the Release of Genetically Modified Organisms for Field Applications

Genetically modified organisms (GMOs) can be produced by introducing the gene of interest into other organisms to accelerate their performance. A variety of GMOs have been developed through genetic engineering and utilized in the degradation and detoxification of organic and inorganic pollutants in lab conditions (Pieper and Reineke 2000; Furukawa 2003; Lovely 2003; Paul et al. 2005).

The introduction of GMOs in field applications may interbreed with the wild type or sexually compatible relatives (Barac et al. 2004). The novel trait may disappear in wild types unless it confers a selective advantage to the recipient. However, tolerance abilities of wild types may also develop, thus altering the native species' ecological relationship and behavior. Faster growth of GMOs can enable them to have a competitive advantage over the native organisms. This may allow them to become invasive, spread into new habitats, and cause ecological and economic damage. Pressure may increase on target and nontarget species to adapt to the introduced changes as if to a geological change or a natural selection pressure causing them to evolve distinct resistant populations. The effects of changes in a single species may extend well beyond to the ecosystem. Single impacts are always joined by the risk of ecosystem damage and destruction. Once the GMOs have been introduced into the environment and some problems arise, it is impossible to eliminate those (Prakash et al. 2011).

One risk of particular concern relating to GMOs is the risk of horizontal gene transfer (HGT). HGT is the acquisition of foreign genes (via transformation, transduction, and conjugation) by organisms in a variety of environmental situations. It occurs especially in response to changing environments and provides organisms, especially prokaryotes, with access to genes other than those that can be inherited (Martin 1999; Ochman et al. 2000; Prakash et al. 2011).

However, to overcome the associated constraint, researchers from around the globe have made several efforts to delimit the uncontrolled proliferations and survival of genetically engineered microbes (GEMs) and stop the horizontal gene transfer (HGT) to the native microbes (Kolata 1985; Atlas 1992; Paul et al. 2005). In addition, many of these risks are identical to those incurred with regard to the introduction of naturally or conventionally bred species (Sayler and Ripp 2000). But still the GMOs are neither safe nor they should be less scrutinized.

7 Conclusion and Future Outlook

Environmental contamination from around the globe has forced the scientific community to think about the environmental sustainability. Environmental sustainability and safety is a major issue in the world due to rapidly increasing pollution that create health hazards and toxicity in the environment. Environmental pollutants (organic and inorganic in nature) can be hazardous to living beings upon exposure and need to be remediated/detoxified using an array of microbes. Being of highly toxic nature, pollutants sometime can inhibit the growth of remediating microbes and, thus, halt the bioremediation processes. Therefore, genetic engineering can be a potential molecular technique to engineer the intended microbes to enhance their catalytic potential for bioremediation of environmental pollutants. However, the potential risks should also be considered before applying genetically engineered microbes in field.

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