A REVIEW ON USE OF GENETICALLY ENGINEERED MICROORGANISMS FOR BIOREMEDIATION OF ENVIRONMENTAL POLLUTANTS AND HEAVY METALS

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ABSTRACT

Bioremediation refers to the use of organisms to remove environmental pollutants. Besides identifying different new plasmids capable of degrading environmental pollutants, many attempts have been taken by the genetic engineers to enhance the bioremediation potential of different microorganisms. *Escherichia coli* was engineered to convert chlorinated solvents such as trichloro ethylene and the highly toxic methyl parathion (pesticide), to non-toxic form. *Escherichia coli* was also engineered to remove radioactive waste (uranium) from environment through precipitation. *Pseudomonas fluorescens, Escherichia coli* and *C. testosterone* were engineered to degrade different Polychlorinated Biphenyls. *Deinococcus geothermalis, Escherichia coli* and *Ralstonia eutropha* were engineered for bioaccumulation of heavy metals to remove it from environment. *Sinorhizobium meliloti* and *Escherichia coli* were engineered for decolorization of azodye. Poly Aromatic Hydrocarbons and 2,4-dichlorophenoxyacetate (pesticide) degrading plasmids have been identified and transferred to indigenous bacteria of polluted soil through bioaugmentation which rendered them capable of degrading respective pollutant effectively.

KEY WORDS: Bioremediation, heavy metals, pollutant, genetic engineering, micro organisms

INTRODUCTION

Due to the various limitations of traditional process of cleaning environmental pollutants, bioremediation obtained gradual attention over last two decade. Extensive research in this field identified many bacterial strains capable of degrading various environmental pollutants. Many catabolic pathways for degrading pollutants have also been identified and explained. However, the degradative capabilities of these strains and pathways are often limited by various factors such as low expression of degradative enzymes, low growth of the bacterial strains in the contaminated environment and inability of these strains to degrade various pollutants by same strain. Various genetic engineering attempts have been taken to overcome these limitations. Plasmids containing biodegradative capabilities have been isolated from parent strains and transformed into bacterial strains which can survive well in the polluted environment. Recombinant bacteria containing several degradative plasmids have also been produced capable of degrading various pollutants simultaneously. The expressions of enzymes involved in catabolic pathway have been increased by various processes including creation of recombinant plasmid. Many bacterial strains have been engineered to express heavy metal transporter or heavy metal sequestering protein for removal of heavy metals from environment. Thus, significant improvement is done in the field of bioremediation using genetically engineered microorganisms.

BIOREMEDIATION OF VARIOUS POLLUTANTS

1. Biodegradation of Petroleum: In 1970s Chakrabarty and colleagues created a bacterial strain capable of degrading different hydrocarbon of petroleum. *Pseudomonas putida* was transformed with four plasmids CAM, OCT, XYL and NAH capable of degrading camphor, octane, xylene and naphthalene respectively. The resulting strain has been called superbug for its increased metabolic capacity. Suparbug can be used to remove oil spill from sea which poses serious health risks to the marine creatures. Most of the engineered microorganisms transformed to confer degradative capability were mesophils, the organism that grow at 20-40^0 centigrade temperature. However the oceans generally have temperature below 20^o centigrade where only psychrotrophic microorganisms can grow. Tolune degrading TOL plasmid from *Pseudomonas putida* PaW1 was transferred into a psychrotrophic bacteria *Pseudomonas putida* Q5, which was capable of degrading salicylate. The resultant strain was capable of degrading both toluene and salicylate at temperature as low as 0^0 centigrade (Kolenc RJ, 1988).

2. Biodegradation of Chlorinated Hydrocarbon (Tri Chloro Ethylene): Chloro ethylenes such as Tri Chloro Ethylene (TCE) are persistent environmental pollutants which is carcinogenic in nature. Toluene dioxygenase (TolDox) in *Pseudomonas putida* found to have catalytic activity against TCE. *Pseudomonas pseudoalcaligenes* KF707 contains a multi-component enzyme Biphenyl dioxygenase (BphDox) which is composed of alpha and

beta subunits, a ferredoxin and a ferredoxin reductase. A recombinant *Escherichia coli* was produced containing BphDox, where alpha subunit of BphDox of *Pseudomonas pseudoalcaligenes* KF707 had been replaced with alpha subunit of toluene dioxygenase from *Pseudomonas putid.* It showed higher catalytic activity toward TCE (Maeda T, 2001). In another study Recombinant *Escherichia Coli* was created by using *Pseudomonas mendocina* as donor. It was capable of reducing the concentration of TCE as much as 100 folds by converting it to chloride ion, CO_2 and water soluble molecule.

3. Biodegradation of PCB: Polychlorinated biphenyls (PCBs) are widely used in different industries. Since PCB's are heat resistant, chemically stable, less soluble in water and highly soluble in lipid, it easily persists in environment. PCBs are found in high concentration in soil around industrial area and used to be removed traditionally by incineration process. Several soil bacteria (such as *Burkholderia* sp. strain LB400) have been found to have enzymes encoded from bph operon which convert PCB to chlorobenzoate and 2-hydroxypenta-2, 4-dienoate. Since *Burkholderia* sp. strain LB400 cannot survive well in natural environment, a derivative of *Pseudomonas fluorescens* F113 has been transformed with bph operon

Table 1-List of Environmental Pollutants (Vidali, 2001) and Respective Genetically Engineered Microorganism for Bioremediation

Class of contaminants	Specific examples	Sources	Microorganism	Reference
Hydrocarbon of Petroleum	Naphthalene, Xylene, Octane, Toluene, Salicylic acid.	Fuel company	*Pseudomonas putida*	(Chakrabarty, 1970) (Kolenc RJ, 1988)
Chlorinated Solvents	Trichloroethylene, Perchloroethylene.	Drycleaners, Chemical manufacture.	*Escherichia coli*	(Maeda T, 2001) (Robert B. Winter, 1989)
Polychlorinated Biphenyls (PCB)	4-Chlorobiphenyl, 4,4-Dichlorobiphenyl.	Electrical manufacturing, Power station, Railway yards.	*Pseudomonas fluorescens, Escherichia coli, C. testosterone.*	(Erickson BD, 1993) (Hrywna Y, 1999)
Pesticides	Atrazine , Carbaryl, Carbofuran, Coumphos, Diazinon, Glycophosphate, Propham, 2,4-D,Parathion.	Agriculture, Timber treatment plants, Pesticide manufacture, Recreational areas, Landfills.	Indigenous bacteria of soil	(Inoue D, 2012) (Li L, 2008)
Radioactive Waste	Uranium	Nuclear reactor	*Escherichia coli*	(Nilgiriwala KS, 2008)
Dye	Azodye	Garment Effluent	*Sinorhizobium meliloti, Escherichia coli.*	(Schlüter A, 2007)
Poly Aromatic Hydrocarbons (PAHs)	Naphthalene, Antracene, Fluorene, Pyrene, Benzo(a)pyrene.	Oil production & storage, Gas work sites, Coke plants, Engine works, Landfills, Tar production & storage oiler ash dump sites, Power stations.	Indigenous bacteria of soil	(Juhasz A. , 1998)
Heavy Metal	Iron, Chromium, Arsenic, Cadmium, Mercury.	Industrial Waste	*Deinococcus geothermalis, Escherichia coli, Ralstonia eutropha.*	(Hassan Brim1, 2003) (Jan Kostal, 2004) (Kim SK, 2005) (Wilson, 1997)

under its own promoter. This bacteria use PCB as sole carbon source and showed greater survival since it resides in rhizosphere. To increase the expression of bph operon, bph operon was cloned under Nod promoters since these promoters have high expression inside *Pseudomonas fluorescens* F113. In a separate study, fusion of Nod promoter created using nodbox 4 alone and the nodD1 from *Sinorhizobium meliloti* resulted in three times higher expression (M. Whelan, 2005). In another study, shuffled bph A1 gene was produced by recombination using *Pseudomonas pseudoalcaligenes* KF707 and *Burkholderia cepacia* LB400. The shuffled bphA1 gene and other genes of bph operon (bphA2A3A4BC) taken from *Pseudomonas pseudoalcaligene* KF707 were used to transform *Escherichia coli* JM109. The recombinant *E. coli* JM109 showed higher degradation of different biphenyl compounds due to the presence of chimeric Biphenyl Dioxygenases (Kumamaru T, 1998). The substrate specificity of PCB was increased when site directed mutagenesis was used to convert bphA of LB400 to the corresponding sequence of KF707 (Erickson BD, 1993). Other than *E. coli, C. testosteroni* was also transformed by using two plasmids pE43 (containing ortho dechlorination *ohb* gene) and pPC3 (containing para dechlorination *fcb* gene) to produce *C. testosterone* VP44 capable of degrading both ortho- and para- chlorinated biphenyl (Hrywna Y, 1999).

4. Biodegradation of Pesticide (2,4 Dichloro phenoxyacetate and Methyl Parathion): 2,4-dichlorophenoxyacetate(2,4-D) is the most widely used herbicide of this world and identified as "possibly carcinogenic to humans" by the International Agency for Research on Cancer (IARC) (Lyon, 1987). *Pseudomonas putida* or *Escherichia coli* contain 2,4-D degradative plasmid pJP4 which was introduced into soil bacteria by bioaugmentation. Gene bioaugmentation is the process of receiving a degradative plasmid by indigenous bacteria in environment from a newly introduced bacteria via dissemination. Bioaugmentation conferred the indigenous bacteria the 2.4-D degradation capability without any irretrievable depressive effects (Inoue D, 2012).

Methyl parathion (MP) is a widely used pesticide which is found to be highly toxic. Parathion disrupts the nervous system by inhibiting acetylcholinesterase (S. Kegley). It is already restricted or banned into many countries. Even treatment of methyl parathion containing water may cause release of it in the environment. *Escherichia coli* BL21 was genetically modified for high express methyl parathion hydrolase, which causes hydrolysis of methyl parathion and release of p-nitrophenol (PNP). A laboratory-scale bioreactor containing genetically modified *Escherichia coli* BL21 and PNP degrading *Ochrobactrum* sp. strain LL-1 caused 98% removal of MP and 100% removal of PNP (Li L, 2008).

5. Bioremediation of Radioactive Waste: Radioactive Uranium is highly toxic to heart, renal system, reproductive system, central nervous system, immune system and DNA (Craft, Abu-Qare, Flaherty, Garofolo, & Rincavage, 2004). phoK gene from *Sphingomonas* sp. strain BSAR-1 was cloned and over expressed in *Escherichia coli* strain BL21(DE3) which showed 13 times higher secretion of alkaline phosphatase in the extracellular medium than BSAR-1 and quickly precipitated more than 90% percent added uranium (Nilgiriwala KS, 2008).

6. Biodegradation of Triphenylmethane Dye: Plasmid pGNB1 confers resistance to the triphenylmethane dyes via its tmr gene observed by the decolorization of the dyes such as crystal violet, malachite green and basic fuchsin to a non-toxic form. This plasmid was found to be transferable to *Sinorhizobium meliloti* and *Escherichia coli*. Since pGNB1 was originally found in activated sludge compartment of a wastewater treatment plant, bacteria transformed with it, can easily be used to treat sewage polluted with triphenylmethane dyes (Schlüter A, 2007).

7. Biodegradation of Poly Aromatic Hydrocarbons (PAHs): Over decades many PAH degrading bacterial strains have been isolated. But these microorganisms are not naturally present in the soil which is highly contaminated with PAHs. Bioaugmentation is the technique that can be used to overcome this limitation. PAH degrading capability can be transferred from the strains found to be capable of degrading PAHs to the soil bacteria which are newly contaminated with PAHs and does not have an adapted microbial population through bioaugmentation (A.L Juhasz, 1996). Five strains of *Burkholderia cepacia* have been found to be capable of using PAHs as sole carbon source (Juhasz A. B., 1997). The biodegradation capability of these strains was successfully transferred to indigenous soil bacteria by bioaugmentation after a lag period (Juhasz A. , 1998). Though PAH degrading pathways are exist in many bacterial strains, inability of PAHs to pass bacterial cell wall limits the degradation of PAHs by these strains.

BIOREMEDIATION OF HEAVY METAL

Deinococcus radiodurans is the best characterized strain of *Deinococcaceae* bacterial family. So it had been transformed with plasmids pMD727 which rendered it capable of degrading various heavy metals. *Deinococcus geothermalis* was isolated from hot spring and was found to be resistant to ionizing radiation and capable of

growing at high temperatures around 55°C (Ferreira, 1997). Where D. radiodurans degrades waste at temperatures less than 39°C and cannot grow in nutritionally restricted environment, D. geothermalis grow in high-temperature and nutritionally restricted environment without exogenous amino acids. So D. geothermalis was transformed with plasmids pMD727 designed for D. radiodurans yielding strain MD865 which showed efficient conversion of heavy metals from toxic to non-toxic form. Following 14 h of incubation with Hg(II) in a microplate at 32°C strain MD865 showed substantial Hg(0) volatilization. It Reduced Fe(III)- nitrilotriacetic acid in the presence of lactate or pyruvate at 45°C, reduced Cr(VI) in under both aerobic and anaerobic conditions at 40°C and reduced U(VI) only in the presence of the AQDS at 40°C. Thus D. geothermalis transformed with pMD727 was metabolically proficient, extremely radiation-resistant, capable of growing at high temperature and efficient for bioremediation of heavy metal & radionuclides (Hassan Brim1, 2003).

Thermus thermophilus HB8 contains arsentie oxidase capable of oxidizing arsenic from toxic to non toxic form. The small and large subunits of arsentie oxidase is encoded by TTHB128 and TTHB127 genes respectively. These genes were cloned into broad-host-range vector pBBR1MCS-5 and it was used to transform various microorganisms rendering them capable of oxidizing 87.6% arsenite (Yang C, 2010). The metallo regulatory protein ArsR has high affinity to arsenite. When it was over expressed in Escherichia coli, increased bioaccumulation of arsenite occurred but the cell growth was reduced. When an elastin-like polypeptide (ELP153) was fused with ArsR, it showed improved cell growth without altering its arsenite bioaccumulation ability.
In total the genetically modified cell accumulated 5- and 60-fold-higher levels of arsenate and arsenite than control cells (Jan Kostal, 2004).

A heavy metal tolerant species Ralstonia eutropha was modified so that it expresses mouse metallothionein on the cell surface. When this was introduced in the soil contaminated with Cd2+, it significantly decreased the toxicity symptom of Cd2+ in model plant (F.Valls M, 2000). An Escherichia coli transformed with a manganese transport gene (mntA) and a metal-sequestering protein (metallothionein or MT) gene showed six time higher accumulation of Cd2+ ion in an aqueous phase than control (Kim SK, 2005).

An Hg2+ transport system and metallothionein was intrudced into Escherichia coli for the bioaccumulation of Hg2+. When glutathione S-transferase fusion protein of Saccharomyces cerevisiae or pea metallothionein was overexpressed in the same cell, it significantly increased the bioaccumulation of Hg2+ and also protected the cell from its harmful effect (Wilson, 1997).

CONCLUSION

During the past 20 years, recombinant DNA techniques have been studied intensively to improve the degradation of hazardous wastes under laboratory conditions. However, relatively few examples of GEM applications in environmental ecosystems exist. Unfortunately, the only manner to fully address the competence of GEMs in bioremediation efforts is long-term field release studies. It is therefore essential to performed field studies to acquire the requisite information for determining the overall effectiveness and risks associated with GEM introduction into natural ecosystems.

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