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8. Role of Genetically Engineered Microbes for Bioremediation of Pollutants in Contaminated Areas

Narayan Sarkar

Phd Research Scholar,
Department of Botany,
Gauhati University.

Barasha Ray

Msc Botany student,
Department of botany,
Gauhati University.

Abstract:

Environmental pollution is a major problem of this century which affects biodiversity, public health and ecosystems worldwide. The conventional technology of solving this pollution problem is not effective and time consuming. This method focusses only on separation of pollutants rather than the degradation of pollutants. The recent technology focuses on the quick degradation of the pollutants. Several genetically engineered micro-organisms (specially bacteria) are able to degrade the environmental pollutants from contaminated sites such as soil, aquatic systems etc. Several bacteria contain plasmids, genes of which can produce enzymes for degradation of toxic chemicals. Different types of bacteria have been developed through recombinant DNA technology and they are utilized for removal of heavy metals and toxic chemicals from contaminated sites. This chapter focuses on how the transgenic micro-organisms are developed by transferring genes by plasmid vectors and also describes the use of different transgenic microbes for the degradation of different pollutants. The chemical reactions involved in degradation of pollutants by the transgenic micro-organisms are also discussed.

Keywords:

Genetically engineered micro-organisms, Recombinant DNA technology, biodiversity, pollutants.

8.1 Introduction:

Bioremediation refers to the process of using microorganisms to remove the environmental pollutants or prevent pollution. The removal of organic wastes by microbes for environmental clean-up is the essence of bioremediation.

The other names used for bioremediation are biotreatment, bio reclamation and bio restoration. Bioremediation refers to the process of using microorganisms to remove the environmental pollutants i.e., the toxic wastes found in soil, water, air etc.

8.1.1 Bioremediation by Genetically Engineered Microbes:

A. Super Bug:

Over the past decade, numerous studies have shown that these facilities leak waste containing antibiotic residue and drug-resistant bacteria into the environment.

This pollution, experts warn, fuels the spread of so-called superbugs, micro-organisms that are resistant to antibiotics. Polluted water bodies like lakes and rivers can be treated with genetically engineered microorganisms (GEMs) A contaminated site, of either terrestrial or aquatic ecosystems, that is polluted with toxic chemicals is deadly for the environment.

The textile, leather, fertilizer and other industries are continuously releasing toxic pollute into our land and rivers, disturbing the normal balance of both the ecosystems which is alarming for a clean and healthy environment.

Although there are various ways to clean up the environment such as recycling the wastes, incineration or disposing the wastes and pollutants into landfill sites, the best and most eco-friendly way to clean up the pollutants is using the microorganisms, the process known as bioremediation. Genetically engineered microbes (GEMs) or the so-called superbugs could be a very promising option to perform this job.

Nature performs its own way of cleaning the environment by biodegradation of the toxic chemicals by its inhabitant microorganisms to maintain a perfect balance.

This process is known as intrinsic bioremediation or bio restoration. But in this modern and industrialized society, the rate of pollution, probably, has gone far beyond what the natural biodegradation can deal with. Moreover, the generation of recalcitrant molecules, chemicals which are hard to degrade, and xenobiotics, unnatural chemical substances in the environment, has made it quite difficult for the natural microorganisms to cope with those pollutants.

However, microorganisms also evolve to gain the capability of degrading certain chemicals. Here comes the opportunity for the biotechnologists to apply a simple trick and what they do is combine several characteristics, capable of degrading different chemicals, from different bacteria into a single one simply by transferring the plasmids responsible for those different characteristics making the new bacterium a superbug.

Plasmids are extra chromosomal genetic elements of bacteria containing certain genetic traits that can hop to other bacteria and gain the capability of reproducing independently into the new ones and share the traits with them.

'Superbug' is a constructed bacterium, hydrocarbons found in petroleum wastes. It is a multi-genetic engineering technique.

Super bug was developed by Anand Chakrabarty spills as a measure to control oil pollution. Petroleum products contain cycloalkenes (octane), napthenes, xylene, tolune and compounds are not easily biodegradable, oil wastes become a major and water. Chakrabarty et al. took attempts to degrade oil wastes using microbes.

They developed superbug to control oil pollution. Prof.Ananda mohan chakraborty et al. (1970) developed and patented a"superbug"that was made to degrade oil and hence can clean up this oils pill over from the sea. *Pseudomonas putida* was transformed with plasmids derived from four different bacteria containing camphor,octane,xylene and napthalene degrading plasmids to make it into a superbug.

So, now the superbug can degrade all the four components alone unlike it's sources which could break down anyone of these four and can be effectively used as agent to clean the oils pills in the ocean.

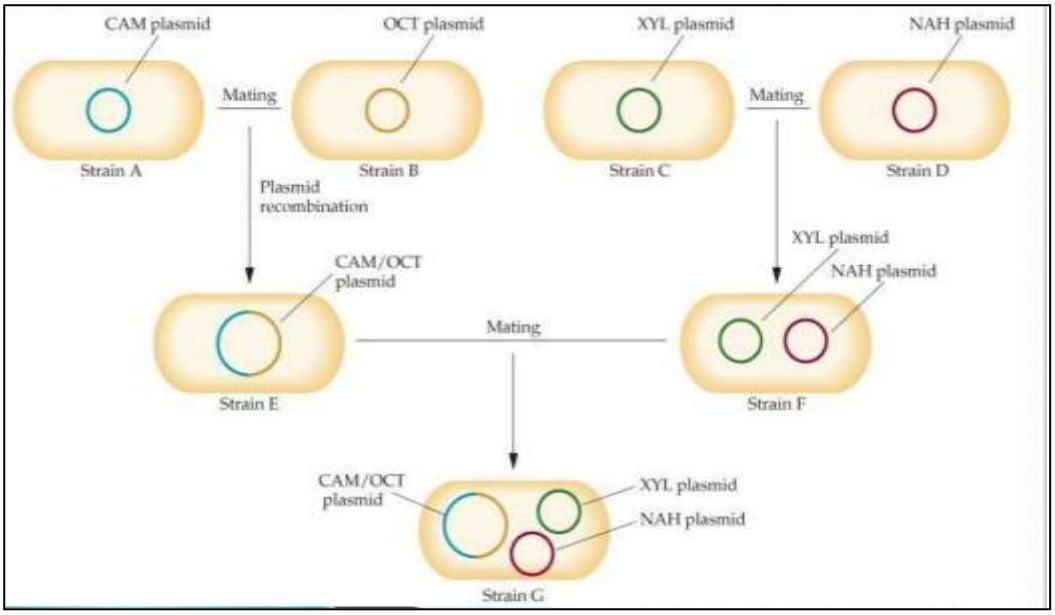

Figure 8.1: Creation of the super bug

8.1.2 Other Genetically Engineered Microbes for Remediation:

Environmental biotechnology indicates that microbes such as bacteria, yeast and filamentous fungi can remove heavy metals from aqueous solutions. The use of microbial metabolic potential is a safe and cost-effective method for the elimination of contaminants from contaminated sites. GE microbes, recombinant DNA and RNA technologies have all been used for successful bioremediation. Microbial genes have been tailored to create new metabolic pathways in order to enhance bioremediative processes. GE microbes may be the preferred technology due to the special attributes of their metabolic pathways [1, 2]. GE bacteria are an advanced technology that has attracted public attention when employed in cleaning up toxic waste and heavy metals from contaminated sites [3,4]. It has also contributed to the detoxification of heavy metals and other recalcitrant compounds [5]. The metal regulatory genes of bacteria can help them to convert toxic forms of heavy metals to less toxic forms [6–9].

GE bacteria expressing metallothioneins (MT) can accelerate the accumulation of heavy metal [10]. Types of GE bacteria involved in the bioremediation of heavy metals from contaminated sites are shown in Table 1.

Mercury is the most toxic heavy metal which can be released into the environment. GE Escherichia coli strain JM109 has the ability to remove mercury from contaminated water, soil or sediment [11]. GE bacteria containing the MerA gene can remove mercury from a contaminated site [12–14]. Transgenic bacteria expressing metallothioneins and polyphosphate kinase can promote effective mercury bioremediation [15].

GE Deinococcus radiodurans and Pseudomonas putidia are capable of degrading organic pollutants in contaminated sites [16]. The use of organophosphates in agriculture, as pesticides, has been shown to cause serious environmental pollution. GE bacteria are capable of metabolizing chlorinated organic compounds such as lindane and trichloroethylene [16].

Lindane (c-hexachlorocyclohexane) is extremely toxic to humans and is deleterious for the environment. Chaurasia et al. [17] reported that the recombinant Anabaena was able to degrade more than 98% of the Lindane from paddy fields within 6–10 days. GE E. coli and P. putida have been found to degrade trichloroethylene, via their transformation through the insertion of a range of phenol catabolic genes (pheA, pheB, pheC, pheD and pheR) [19]. Marconi et al. [18] reported that GE P. putida S12 can degrade naphthalene, toluene and biphenyl following insertion of the plasmids encoding genes for the catabolism of thesecontaminants.

Table 8.1: Genetically Engineered Bacteria Involved for Bioremediation of Heavy Metals

Genetically engineered bacteria species	**Targeted heavy metal**	**References**
Ralstonia eutropha CH34	Cd	[20]
Deinococcus radiodurans strains	Hg	[21]
Escherichia coli and Moraxella sp.	Cd and Hg	[22, 23]
E. coli strain	Hg	[24]
P. fluorescens 4F39	Ni	[25]
Mesorhizobium huakuii B3	Cd	[26]

Genetically engineered bacteria species	Targeted heavy metal	References
P. putida strain	Cr	[27]
E. coli SE5000	Ni	[28]
E. coli JM109	Hg	[29]
Acidithiobacillus ferrooxidans strain	Hg	[30]
Pseudomonas K-62 Pseudomonas fluorescens OS8	Hg	[31]
Escherichia coli MC1061; Bacillus subtilis BR151; Staphylococcus aureus RN4220	Cd, Zn, Hg and Pb	[32]
P. putida 06909	Cd	[33]
Achromobacter sp AO22	Hg	[34]
Methylococcus capsulatus (Bath)	Cr	[35]
Caulobacter crescentus JS4022/p723-6H	Cd	[36]
Sphingomonas desiccabilis and Bacillus idriensis strains	As	[37]
B. subtilis BR151 (pTOO24)	Cd	[38]

8.2 Consortia of Microorganisms for Biodegradation:

A particular strain of microorganism may degrade one or more compounds. Sometimes, for the degradation of a single compound, the synergetic action of a few microorganisms (i.e. a consortium or cocktail of microbes) may be more efficient. For instance, the insecticide parathion is more efficiently degraded by the combined action of Pseudomonas aeruginosa and Psudomonas stulzeri. Members of the genus Pseudomonas (a soil microorganism) are the most predominant microorganisms that degrade xenobiotic.

Different strains of Pseudomonas, that are capable of detoxifying more than 100 organic compounds, have been identified. The examples of organic compounds are several hydrocarbons, phenols, organophosphates, polychlorinated biphenyls (PCBs) and polycylic aromatics and naphthalene. About 40-50 microbial strains of microorganisms, capable of degrading xenobiotics have been isolated. Besides Pseudomonas, other good examples are

Mycobacterium, Alcaligenes, and Nocardia. A selected list of microorganisms and the xenobiotics degraded is given in Table 8.2.

Table 8.2: A selected List of Microorganisms and The Xenobiotics Degraded

A selected list of microorganisms and the pollutants (xenobiotics) that are degraded by bioremediation

Microorganism	*Pollutant chemicals*
Pseudomonas sp	Aliphatic and aromatic hydrocarbons—alkylaminoxides, alkylammonium benzene, naphthalene, anthracene xylene, toluene, polychlorinated biphenyls (PCBs), malathion, parathion, organophosphates.
Mycobacterium sp	Benzene, branched hydrocarbons, cycloparaffins
Alcaligenes sp	Polychlorinated biphenyls, alkyl benzene, halogenated hydrocarbons.
Nocardia sp	Naphthalene, alkylbenzenes, phenoxyacetate.
Arthrobacter sp	Benzene, polycyclic aromatics, phenoxyacetate, pentachlorophenol.
Corynebacterium sp	Halogenated hydrocarbons, phenoxyacetate.
Bacillus sp	Long chain alkanes, phenylurea.
Candida sp	Polychlorinated biphenyls
Aspergillus sp	Phenols
Xanthomonas sp	Polycyclic hydrocarbons
Streptomyces sp	Halogenated hydrocarbons, phenoxyacetate.

8.3. Enzyme Systems for Biodegradation:

Several enzyme systems (with independent enzymes that work together) are in existence in the microorganisms for the degradation of xenobiotics.

The genes coding for the enzymes of bio-degradative pathways may be present in the chromosomal DNA or more frequently on the plasmids. In certain microorganisms, the genes of both chromosome and plasmid contribute for the enzymes of biodegradation.

The microorganism Pseudomonas occupies a special place in biodegradation.

List of Xenobiotics and Plasmids:

Table 8.3: List of Xenobiotics and plasmids

Xenobiotic	*Name of plasmid in* Pseudomonas
Naphthalene	NAH
Xylene	XYL
Xylene and toluene	TOL, pWWO, XYL-K
Salicylate	SAL
Camphor	CAM
3-Chlorobenzene	pAC25

8.4 Metabolic Effects of Microorganisms on Xenobiotics:

Although it is the intention of the biotechnologist to degrade the xenobiotics by microorganisms to the advantage of environment and ecosystem, it is not always possible. This is evident from the different types of metabolic effects as shown below.

A. Detoxification: This process involves the microbial conversion of toxic compound to a nontoxic one. Biodegradation involving detoxification is highly advantageous to the environment and population.

B. Activation: Certain xenobiotics which are not toxic or less toxic may be converted to toxic or more toxic products. This is dangerous.

C. Degradation: The complex compounds are degraded to simpler products which are generally harmless.

D. Conjugation: The process of conjugation may involve the conversion of xenobiotics to more complex. compounds. This is however, not very common.

8.5 Types of Reactions in Bioremediation:

Microbial degradation of organic compounds primarily involves aerobic, anaerobic and sequential degradation.

8.5.1 Aerobic Bioremediation:

Aerobic biodegradation involves the utilization of O2 for the oxidation of organic compounds. These compounds may serve as substrates for the supply of carbon and energy to the microorganisms.

Two types of enzymes namely mono-oxygenases and di-oxygenases are involved in aerobic biodegradation.

Monooxygenase can act on both aliphatic and aromatic compounds while di-oxygenase oxidize aliphatic compounds.

8.5.2 Anaerobic Bioremediation:

Anaerobic biodegradation does not require O2 supply. The growth of anaerobic microorganisms (mostly found in solids and sediments), and consequently the degradation processes are slow.

However, anaerobic biodegradation is cost- effective, since the need for continuous O2 supply is not there. Some of the important anaerobic reactions and examples of organic compounds degraded are listed below.

Hydrogenation and dehydrogenation — benzoate, phenol, catechol.

Dehaiogenation — Polychlorinated biphenyls (PCBs), chlorinated ethylene's. The term de -chlorination is frequently used for dehaiogenation of chlorinated compounds.

Carboxylation and decarboxylation — toluene, cresol and benzoate.

8.5.3 Sequential Bioremediation:

In the degradation of several xenobiotics, both aerobic and anaerobic processes are involved. This is often an effective way of reducing the toxicity of a pollutant. For instance, tetra chloromethane and tetrachloroethane undergo sequential degradation.

A. Biodegradation of Hydrocarbons: Hydrocarbons are mainly the pollutants from oil refineries and oil spills. These pollutants can be degraded by a consortium or cocktail of microorganisms e.g., Pseudomonas, Corynebacterium, Arthrobacter, Mycobacterium and Nocardia.

B. Biodegradation of Aliphatic Hydrocarbons: The uptake of aliphatic hydrocarbons is a slow process due to their low solubility in aqueous medium. Both aerobic and anaerobic processes are operative for the degradation of aliphatic hydrocarbons. For instance, unsaturated hydrocarbons are degraded in both anaerobic and aerobic environments, while saturated ones are degraded by aerobic process. Some aliphatic hydrocarbons which are recalcitrant to aerobic process are effectively degraded in anaerobic environment e.g. chlorinated aliphatic compounds (carbon tetrachloride, methyl chloride, vinyl chloride.

C. Biodegradation of Aromatic Hydrocarbons: Microbial degradation of aromatic hydrocarbons occurs through aerobic and anaerobic processes. The most important microorganism that participates in these processes is Pseudomonas. The biodegradation of aromatic compounds basically involves the following sequence of reactions:

A. Removal of the side chains.

B. Opening of the benzene ring.

8.6 Conclusion and Future Perspectives:

Among the top ten biotechnologies for improving human health, bioremediation is recognized as one of the technologies (Eapen et al. 2007). The application of molecular-biology-based techniques in bioremediation is being increasingly used and has provided useful information for improving of bioremediation strategies. Furthermore, environmental metagenomic data from soil and sea can be a useful source of genes. Combinational approaches such as genome shuffling are also useful for generating new genes or modifying enzyme activities to allow efficient bioremediation (Kawahigashi 2009).

This new biotechnology approach will open exciting new vistas for enhancing bioremediation programs in the coming years.

Transgenic strategies have created enormous opportunities for the removal of contaminants within the environment. This technology is environmentally sound and cost effective compared to conventional technologies. Environmental factors that can influence the biodegradation of contaminated sites should also be taken into account. The safe use and containment of GEO, with proper regulatory procedures, for bioremediation should also be practiced. GEO may be a suitable and sustainable method for the bioremediation of contaminated sites but there are some current challenges such as the dispersal of transgenic pollen, the horizontal transfer of plasmids amongst microbes and the poor survival of GEO and transgenic plants. Researchers should focus on the development of more suitable technologies using self-pollinated transgenic plants and creating infertile polyploidy in strains. Equally, the development of combined strategies for bioremediation using GEO has the potential to overcome current challenges.

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