Bioremediation of radioactive waste from the sea

Abstract:

Thousands of waste sites around the world contain non-degradable radioactive material. A lot of danger and expense of cleaning up such wastes by physicochemical methods and hence the other methods are being pursued for cleanup of those sites. One effective method is to engineer radiation-resistance microbes that degrade or transform such wastes into less hazardous compounds. Deinococcus radiodurans, the most radiation-resistance organism ever known and endure 1, 500 Kilorad/hr gamma or UV radiation and grow in radioactive environment. This characteristic is useful for remediation of radioactive waste material by introducing the gene from Pseudomonas putida spp. into Deinococcus radiodurans genome (strain R1-ATCC BAA-816) which is responsible for degradation. This recombinant strain will degrade the harmful radioactive material and clean up the sea environment by converting into less harmful compounds.

Introduction and Background:

Nuclear weapon production in earlier 5 decades has generated most of the wastes which was directly discharged into the water and now are contaminating thousands of sites. In the United States, about one third of the reported waste sites are radioactive, with radiation levels as high as 10 mCi/L in or close to the contaminating sources (14). These highly toxic wastes contain inorganic and organic contaminants that include radionuclide such as 235Uranium, heavy metals such as mercury, and solvents such as toluene (14).

There is little prospect for cleanup of these waste sites by physicochemical method alone because of extreme expense, danger and intensity of labor.

The clean-up cost of these waste sites by physicochemical method was estimated to be $90 billion 1988 and more recently to be about $265 billion (15). Unless new costs effective clean up technologies are developed, theses waste increasing will threaten to the marine life and humans.

Numerous organisms (particularly Pseudomonas sp.) have been described that have the ability to degrade, transform and detoxify the organic and inorganic pollutants (16-21). Most of the micro-organisms are sensitive to the damaging effect of radiation found in the radioactive waste and are not suited for remediation of radioactive waste material. Therefore, radiation-resistant micro-organisms that can degrade radioactive waste material need to be found in nature or engineered in the laboratory to solve this problem.

The most radiation resistance organism is discovered till now is Deinococcus radiodurans (Figure-1) (22-23). It is pink is color because of carotenoid pigment present in it. It consists of 4-10 identical copies of a chromosome (2. 65 Mbp), two megaplasmid (412 and 177 kbp) and a plasmid (46 kbp) (4-6). This is a non-pathogenic, spherical shaped, Gram Positive aerobic bacteria bacterium that can grow continuously in the presence of Ì´ 1, 500 Kilorad/hr Gamma or UV radiation with no effect on either its growth rate or its ability to express foreign genes (27).

Deinococcus radiodurans bacterium has the capability to grow in the radioactive environment. This property is very useful for bioremediation of radioactive substances by incorporating genes from the Pseudomonas putida sp. The ability of a microorganism to remediate the radioactive waste

material is associated with the ability to transform these materials into less harmful compounds.

Toluene is water-insoluble liquid of benzene derivative. It is an aromatic hydrocarbon and if inhaled in large amount, it causes neurological harm which may lead to death also. When it is mixed in the water, it is difficult to degrade toluene. A Deinococcus radiodurans bacterium does not have a gene to degrade toluene. So, when this bacterium is engineered by adding TOD gene from E. coli sp., it is able to degrade toluene (1).

Metals like Hg, in ionic are very toxic. When Hg (II) is mixed with water it takes lot of time to degrade naturally. So, when the gene responsible for degrading ionic Hg (II) that is Hg(II) resistance gene (merA) from E. coli strain BL308 into Deinococcus radiodurans, it resist the toxic effect of the metal and transform those metals to less toxic and less soluble chemical states(2).

IR resistant bacteria Deinococcus radiodurans have high intracellular concentration of Mn/Fe ratio. When high level of radiation exposed to the bacterium, its primary target of biological action is on protein not on DNA. Mn(II) ions are distributed all over the cell but Fe is present in a region where cell division takes place. Mn(II) ions protects protein from oxidative modifications by introducing carbonyl group into it (9-10).

The most studied of Deinococcus sp. is D. radiodurans. Unlike other species of deinobacteria, it is most liable and comfortable to genetic manipulation due to its natural transformability by both high molecular weight chromosomal DNA and plasmid DNA (28-29). The natural ability of transformation of this bacterium has enlightened the many different

techniques for genetic alternation enabling it highly for molecular investigation (29-31).

The aim of this project is to engineer a strain of D. radiodurans that are capable of degrading radioactive waste material. Well known organism those are capable of degrading wastes are not able to survive in these sites because of their sensitivity to the radiations. Generally, most of the organisms are sensitive to the damaging effects of ionizing radiation and most of the bacteria currently being studied are not acceptable to remediate radioactive waste. For example, Pseudomonas sp. is very sensitive to radiation (more sensitive than E. coli) and not suited to remediate radioactive waste. Therefore, radiation resistant organisms are needed to be found in the nature or engineered in the laboratory that can remediate radioactive wastes.

Statement of Research Objectives, Methods and Significance:

The main objectives of this project are:

- To isolate the gene of interest.
- To introduce the desired gene into D. radiodurans.
- To study the expression in D. radiodurans.
- To optimize the media composition for better results.

My working hypothesis is that a Deinococcus radiodurans bacterium grows in radioactive environment. This can be used for bioremediation of radioactive substances. It would be making possible by incorporating genes responsible for degradation of waste material from Pseudomonas putida spp.

Goal 1 –D. radiodurans will be grown on TYG broth medium and P. putida on Basal Salt Medium. Chromosomal DNA is isolated and plasmid DNA is isolated from P. putida (36). Restriction digestion of plasmid DNA and Chromosomal DNA will be done to cut the specific region of DNA (37). Electrophoresis of restriction digested DNA will be the next step to check their DNA is cut at the specific site. Plasmid DNA will be extracted by using electro-elution. Centrifuge the eluted DNA with dialysis buffer which will remove contaminated agarose particles.

Goal 2 –A clone will be prepared by introducing the gene from P. putida to D. radiodurans by transformation. pl3 shuttle vector is used for transformation (32).

Horizontal gene transfer can be done in various ways like transduction, bacterial conjugation and gene transfer agents (35). But transformation is the prominent method to transfer a gene from one microorganism to another microorganism.

Goal 3 –Once the gene is transferred to D. radiodurans from P. putida, screening will be the next step. It will be done by two methods-Gel Shift and Colony PCR. Gel shift (EMSA- Electrophoretic Mobility Shift Assay) method will determine the difference between the transformed DNA and non-transformed DNA. Transformed DNA will run slowly on gel because of its high molecular weight than the non-transformed DNA. By comparing with the marker DNA, it will be easy to visualize the differences. Colony PCR method determines the insert size or orientation in the vector. If the insert is present, the size of the vector will increase. This can be determined by growing each

colony in the liquid medium and plasmid then purified by rapid boiling method digestion by restriction enzymes that excise the insert, followed by separation on agarose gel electrophoresis.

Goal 4 –When a cloned D. radiodurans is ready, media optimization will be crucial for faster growth of the cloned copies. Radioactive material concentration will be changed from lower to higher concentration. Higher and lower glucose concentration will be changed for better results. Some other elements will be added if required.

Future Research Directions

A long term goal of this project is to engineer the cloned copied of D. radiodurans genetically so that it will overexpress. However, to overexpress the cloned copies, first the complete gene sequence, promoters and regulators must be identified and understood. If the results are not satisfactorily good, use different organisms instead of P. putida. Actual field trial of this organism and if it is successful large scale production of this organism would be the last step.