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Study of Radioresistance and Bioremediation using Radio-Resistant Bacteria

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Abstract: ***Nature has always produced variety of life forms, adapted to survive in varieties of environmental conditions. Even in harshest of conditions, where most of the life forms are thought to be killed, life still exists. While exploring these 'extremophiles', a number of living organisms have been found to be excellently radio-tolerant. Their existence and their survival mechanism are of great significance as it paves the way for their application in various biotechnological aspects including bioremediation of nuclear pollutants. In this review, we shall try to put focus on origin and diversity of radiation-resistant bacteria as well as the various selective mechanisms by which microorganisms can sustain in radiation rich environment. Also, we shall try to spot out how these radioresistant bacteria help in nuclear waste clean-up, as they cannot be mobilized easily, and also, they have a long decay period, which makes them difficult to remediate where radionuclides (particularly plutonium and uranium) have been used and/or produced as a by-product.***

Keywords: Bioremediation, Bioactive compound, Extremophiles, Radiation, Radionuclides, Radiation-resistance bacteria

1. Introduction

Microbes have been living in our home planet for millions and billions of years. They've survived nature's onslaughts in various forms such as radiation, temperature, PH abnormalities, and much more. But as the harsh and extreme conditions calmed down microbes have evolved to survive in such type of environment. Still there is a class of microorganism still surviving with the characteristics that could have helped them to survive in such harsh conditions. *Deinococcus radiodurans* is one such microbe that can survive lethal dose of radiation. This type of microbes can survive these conditions, thanks to their ability to repair their DNA with the help of some novel enzymes as well as some novel compounds that help them to protect the cell from any damage, like high radiation. But the strange fact is that resistance to chronic radiation is not limited to rare specialized species or strains from extreme environments; it has occurred among various known microbial taxa (Nayak et al., 2021). It might have happened due to overlap of molecular mechanisms of resistance to radiation and other stressors. The inherent stress tolerance capabilities of these organisms render them promising candidates for the remediation of radionuclides. Furthermore, their extremolytes possess valuable properties that can be harnessed as antioxidants and anti-proliferative agents. In present scenario they can be useful in various fields-from natural dye synthesis to nanoparticles production and anti-cancer treatment (Nayak et al., 2021).

Radioresistant Bacteria:

Many organisms under the three domains of life (Bacteria, Archaea, & Eukarya can tolerate extreme environmental conditions like extreme temperatures, high salt level, high pressure, acidic or alkali conditions, and even, radiation (a. Kumar et al., 2010; Marques, 2018; Orellana et al., 2018). Among these extremophiles, the organisms that are very much resistant to radiation are called radioresistant extremophiles. They are found in many parts of nature; from radioactive contaminated regions (like the area around Chernobyl nuclear reactor), higher elevations (mountain ranges) to open fields where UVR levels are high, desert, etc. (Nayak et al., 2021). The amount of both ionizing and non-ionising radiation that they can tolerate could have easily killed any other form of life. For instance, a bacterium, *Deinococcus radiodurans* can tolerate 15kGy of ionizing radiation, and UV radiation >1000 J/m² (a. Yuan et al., 2009a; b). Near Chernobyl nuclear reactor, four outcomes of radiation exposure of cells have been found, either they are unharmed, killed, survive with DNA damage or survive with radiation-induced genomic instability (b.15-19). Rhodanobacter sp. and *Desulfuromonas ferrireducens* can survive high amounts of radionuclides through the five fundamental processes, namely; biotransformation, bioaccumulation, Biosorption, bioprecipitation and biosolubilisation. (a. Lopez-Fernandez et al., 2020; Shukla et al., 2017).

Diversity of radiation resistant bacteria:

Organisms	Radiation	Environment	Reference
Cellulosimicrobium cellulans UVP1	UVR-type C	Elevated land	Gabani et al, (2012)
Bacillus circulans BR11	Gamma	Radon contaminated groundwater	a. Nayak et al, (2019)
Bacillus altitudinis BR1			
Bacillus altitudinis BR2			
Bacillus altitudinis BR3			
Bacillus altitudinis BR10			
Bacillus altitudinis BR16			
Bacillus altitudinis BR11			

Organisms	Radiation	Environment	Reference
Bacillus altitudinis BR16			
Bacillus cereus BR15			
Bacillus altitudinis BR16			
Deinococcus radiodurans R1	X-ray, UVR and gamma	Canned meat	a. Shukla et al. (2007)
Rubrobacter radiotolerance	Gamma	Unknown	a. Terato et al. (2011)
Deinococcus cellulosilyticus 5516J-15T	UVR and gamma	Air sample, Jeju Island, Korea	a. Weon et al. (2007)
Deinococcus deserti VCD115T	UVR and gamma	Sahara Desert, Morocco	a. de Groot et al. (2005)
Sphingomonas sp. RB2256	UVR-type B	Unknown	a. Gabani and Singh (2013)
Chroococcidiopsis sp.	X-ray	Desert and hypersaline	a. Singh and Gabani (2011)
Halobacterium salinarum	X-ray	Unknown	a. Robinson et al. (2011)
Hymenobacter xinjiangensis	UVR and gamma	Desert	a. Zhang et al. (2007b)
Staphylococcus saprophyticus	UVR	Lake	a. Zenoff et al. (2006)
Acinetobacter sp. Ver3	UVR-type B	Lake	a. Di Capua et al. (2011)
Streptomyces radiopugnans	Gamma	Radiation–pollution	a. Mao et al. (2007)
Deinococcus soli ZLM-202T	UVR and gamma	Arid soil, Xinjiang, China	a. Zhang et al. (2011)
Deinococcus depolymerans TDMA-24T	UVR and gamma	Fresh water, Misasa, Japan	a. Asker et al. (2011)
Deinococcus humi MK03T	UVR and gamma	Soil, Seoul City, South Korea	a. Srinivasan et al. (2012b)
Lysobacter bugurensis ZLD-29T	Gamma	Forest Xinjiang, China	a. Yu et al, (2013)
Deinococcus daejeonensis MJ27T	UVR and gamma	Daejeon sewage, South Korea	a. Srinivasan et al. (2012a)
Deinococcus peraridilitoris KR-200T	UVR and gamma	Coastal desert, Chile	a. Rainey et al. (2007)
Lysobacter bugurensis ZLD-29T	Gamma	Forest Xinjiang, China	a. Yu et al, (2013)
Deinococcus deserti VCD115T	UVR and gamma	Sahara Desert, Morocco	a. de Groot et al. (2005)
Halobacterium salinarum	X-ray	Unknown	a. Robinson et al. (2011)

Mechanism of Radiotolerance

The ability of the radio-tolerant microorganisms to survive high amounts of radiation has been linked to-i) their efficient DNA repair mechanisms, ii) their ability to produce protective primary and secondary metabolic products, i.e., extremolytes and extremozymes (Nayak et al., 2021). Porphyra-334, phlorotannin, scytonemin, shinorine, mycosporine-like amino acids, palythine, and biopterin, etc are some common extremolytes that provides the extremophiles the ability to absorb wide range of radiations and their effectors molecules. These extremolytes have gained significant traction in the field of biotechnology and find diverse applications in commercial sectors including antioxidant formulations, anticancer therapeutics, natural pigments, cell-cycle inhibitors, sunscreens, and nanoparticle synthesis. (Nayak et al., 2021).

It is incredibly captivating to delve into the alterations in the genomics, proteomics, and metabolic profiles of radiation-resistant organisms when subjected to radiation. This exploration allows us to uncover valuable insights into the mechanisms employed by these organisms to endure such conditions. Extensive documentation reveals that the expression of RadB and RadA in Sulfolobus tokodaii significantly increased in the presence of UV radiation compared to their baseline levels. (a. Sheng et al., 2008). *Dictyostelium discoideum* can survive extremely high doses of radiation and DNA cross linking agents due to the presence of the nucleotide excision repair, Fanconi anemia pathway (FA) and translesion synthesis (TLS) (a. Zhang et al.2009). Gamma radiation resistant *Bacillus* sp. HKG 112, after radiation exposure, expresses two proteins, flagellin and S-layer protein showed significant changes (a. Kumar et al., 2011). Studies revealed that *Cellulosimicrobium cellulans* (UVP1) and *Bacillus pumilus* (UVP4), when grown under radiation, confirm differential expression of many previously unknown proteins and metabolites (a. Gabani et al., 2012). *Deinococcus sp.* stands out as the most extensively studied microorganism at the molecular level, unveiling a multitude of novel extremolytes and extremozymes / proteins. (a. Coker, 2019; Joshi et al., 2020; Liedert et al., 2010; Ranawat and Rawat, 2017). Notably, significant attention has been given to exploring potentially novel proteins within the nucleoids of radio resistant microorganisms. In a comparative proteomics analysis between D. radiodurans and D. deserti, it was revealed that the histone-like DNA binding protein (HU) stands as the most abundant protein among the nucleoid-associated proteins. (Nayak et al., 2021) . It also revealed that when Xpf nuclease is disrupted in *D. discoideum*, it resulted in extreme hypersensitivity to crosslinks and radiation, function with FA and TLS gene products (a. Zhang et al., 2007c). Different types of highly efficient DNA repair proteins/enzyme in *D. radiodurans* helps it to repair hundreds of double-stranded DNA breaks. On deletion of a novel polymerase, X family DNA polymerase, there appeared a decrement in the rate of repair of double-stranded DNA breaks and an increase in sensitivity to gamma radiation (a. Leulliot et al., 2009). Further, the increased expression of DR1199 (it is a general stress protein in *D. radiodurans*) may be involved in the detoxifying the cell from reactive oxygen species (ROS) (a. Leulliot et al., 2009).34 abundant proteins with no known function were differentially expressed by *D. geothermalis*; these may have a relation to the extreme stress tolerance of this organism (a. Liedert et al., 2010). D. radiodurans harbors two genes, LexA1 and LexA2, which encode transcriptional regulators involved in the DNA damage response under severe conditions. Interestingly, when the LexA2 gene was disrupted, it led to the activation of the pprA promoter. This activation, in turn, resulted in an increase in the expression of a novel radiation-inducible protein called PprA. (a. Singh and Gabani, 2011). Extensive research has demonstrated that in the case of D. radiodurans, a range of homologous proteins including UvrA (DR1771), UvrB (DR2275), UvrC (Dr1354), and UvrD (DR1775) contribute to protection against UV-induced damage. Additionally, proteins such as recA (DR2340), recF (DR1089), recJ (DR1126), recO (DR0819), and ruvB (DR0596) play important roles in recombination repair of single and/or double-strand DNA damage. The mutS protein (DR1039) is involved in mismatch repair, while superoxide dismutase (SOD) (DR1279) and catalase (DR1998) provide protection against oxidative stress. The combined action of these proteins confers D. radiodurans with its remarkable survival capabilities under radiation-induced stress. (Nayak et al., 2021).

Bioremediation

In a broader sense, bioremediation refers to removal of environmental pollutants (especially heavy metals) from air, water, soil, industrial effluents etc., in natural or artificial settings by employing any biological resource (typically bacteria, algae, fungi, and even plants), living or dead. (c. Meena Kapahi, Sarita Sachdeva; 2019). The ability of the organisms by which they can absorb, accumulate, and degrade common and emerging pollutants has attracted researchers in recent years to use biological systems in treating contaminated environments. Pollution associated with radiation (from radioactive elements like, plutonium, uranium, thorium, radon etc) has become a seriously increasing threat because it cannot be mobilized easily, and it has a long decay period, which makes it difficult to remediate (a. Jin et al., 2019; Lopez-Fernandez et al., 2020). Furthermore, radioactive contamination due to anthropogenic causes also occurs during-ore extraction, nuclear energy generation, and atomic weapon production where radionuclides (particularly plutonium and uranium, in this case) have been used and/or produced as a byproduct (a. Lopez-Fernandez et al., 2020).

Radioresistant Bacteria in bioremediation

Microorganisms play a crucial role in the bioremediation of radioactive contaminants, employing various strategies to facilitate their removal from the environment. These strategies can be broadly categorized into three mechanisms: extracellular enrichment and precipitation, transformation via cell surface adsorption or precipitation, and transformation through intracellular adsorption or precipitation. Through these processes, microorganisms contribute to the reduction and immobilization of radioactive contaminants, aiding in the restoration and cleanup of contaminated areas. (a. Lopez-Fernandez et al., 2020; Shukla et al., 2017). Among many microorganisms *Deinococcus* seems to be more efficient in remediating radionuclides pollution because of its high resistance, tolerance to radionuclide ions absorption and degradation ability. A *Deinococcus*DR1 strain has been isolated, which is radiation and arsenic resistant. It has heavy metal translocation P-type ATPase, heavy metal transport/ detoxification proteins, heavy metal-related domain proteins, and, arsB, arsR and arsenate reductase genes, which provide active resistance against arsenic and radiation (a. Chauhan et al., 2017). Deinococcus radiodurans has demonstrated an impressive capability to eliminate radionuclides such as uranium (VI) with a removal rate of 90% and cobalt (60Co) with a removal rate of 60%. Additionally, Micrococcus luteus, another radiation-resistant strain, has shown effective removal of cadmium (Cd (II)) at a rate of 444 ± 15 µmol/g. (a. Gogada et al., 2015; MacHalov´a et al., 2015; Misra et al., 2012). When genetically engineered, *D. radiodurans* R1 strain has shown expression of synthetic phytochelatin (EC20) and cyanobacterial metallothionein (smtA) genes, that was found to enhance its tolerance and bioaccumulation of Cd^{2+} (a. Chaturvedi and Archana, 2014). *Shewanella putrefaciens* and *Geobacter sulfurreducens*, which have enzyme c type cytochrome, has been found to reduce soluble uranium radioisotopes into insoluble ones (Nayak et al., 2021). *Desulfovibrio desulfuricans* contains a Ni–Fe hydrogenase, that was able to reduce Tc (VIII) (a. Barton et al., 2007). The microbial communities, that are native to radio-contaminated zones, have been used to restore such areas by natural attenuation through biostimulation and/or bioaugmentation (a. Shukla et al., 2017). The increment of *Geobacter* sp. activity caused due to acetate enrichment (biostimulation) had shown enhancement in reduction of uranium, thus leading to successful in situ-based bioremediation (a. Marques, 2018). Nonetheless, soon, metabolic flux modelling should be applied to create novel extremophilic radio resistant bacteria for metals and radionuclides clean-up (a. Hold et al., 2009).

2. Conclusion/ Future direction

More study and research on radioresistant, radio-resistant microorganisms might pave the way to their contribution in biotechnology and human welfare. Such microbes can be very useful for the entire human civilization— starting from cleaning radioactive contamination or any nuclear fallout, to production of novel medicines and many life-saving drugs. The way they tolerate some of the extreme conditions of nature makes us dive deeper and get deep insights on their genetic make-up, molecular interactions and complex protein functions (that are still unknown). The aim of this review has been to focus on the microbial diversity and activity of radioresistant extremophiles and knowledge of their evolution helps us to explore the evolutionary process, genetic and molecular consequences of extreme environments. Besides that, our study also casts light on the importance of radiation-resistant extremophiles in bioremediation.

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