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Review of anticancer and antioxidant activities of radioresistant extremophiles at molecular level: an itinerary to the discovery of cancer drugs in Nigerian extreme radiation environments

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Abstract

Radiation extremophiles exhibits extraordinary resistance to ionizing radiation (electromagnetic or corpuscular). *Chroococcidiopsis sp., Deinococcus radiodurans, Rubrobacter radiotolerans,* and *Thermococcus gammatolerans* are examples of radioresistant microorganisms with the ability to survive and grow under high doses of radiation. Most radioresistant organisms use a combination of repair and protection based mechanisms to achieve high radioresistance. This article emphasizes the molecular mechanism underlying the tolerance of these organisms to ionizing radiation. The procedure applied in molecular cancer therapy such as anticancer drug, antioxidation, and sunscreen ability was discussed. These processes may provide some insight into response of the microorganism's internal processes under different conditions. The developmental process counts on the economic base of the biotechnological industries and their curiosity for molecular level innovative concept from extremophiles. The stimulating test of abilities and future visions of this concept are also mentioned.

Key words: ionizing radiation, extremophiles, radioresistant, extremozymes, extremolytes.

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Introduction

There is a great deal of questions and literatures on the prospects of radioresistant extremophiles in the world, but assessment of this component of knowledge has received little or no attention in Nigeria and Africa at large. A quarter of a century ago, the word extremophile was coined (MacElroy, 1974). It has been defined in a number of ways, and has appeared to be associated to those microorganisms that inhabit environments which other creatures found uninhabitable. Radioresistant extremophiles have the ability to survive high doses of radiation with an essential environmental inert condition for survival which is detrimental to other organism. Extremophiles cut across all three domains of life: bacteria, archaea and eukaryotes, and an intense exposure to radiation (e.g. UV radiation, X-rays, gamma rays, etc) can induce a variety of mutagenic and cytotoxic DNA lesions, which can lead to different forms of cancer. Nevertheless, microorganisms survive by means of metabolic products referred to as extremolytes and extremozymes (Gabani and Singh, 2013). These metabolic products are capable of absorbing a broad spectrum of radiation while protecting the organisms DNA from being damaged (Gabani and Singh, 2013; MacElroy, 1974). The importance of extremophiles does not support only the basics of biochemical and structural biodiversity but, also, their enormous potential as sources of enzymes and other biological materials with applications in biotechnology companies around the world (Jane and Alan, 2004; Podar and Reysenbach, 2006).

The discovery of extremophiles has enabled industry to innovate corresponding bioproducts, extremolytes and extremozymes, for the benefit of man. Many of such processes utilize microorganisms that have means of resisting the effects of ionizing radiation. Advances in understanding the specific functions of microorganisms in such processes, together with the ability to fine-tune their activities using the tools of molecular biology, has led to the development of novel or improved drugs. The aim of this article is to provide an overview of the mechanisms by which microorganisms interact with ionizing radiations, and to highlight the application of these processes to the development of drugs. To also call on researchers, especially the radiation-biophysicists in this part of the world to invest in this kind of research in order affect the life of our people.

Radioresistant Extremophiles – the route for the discovery of cancer drugs: Nature has devised means for microorganisms to strive in an extreme radiation environment where mankind found to be hostile. This has contributed to the development of relatively new and largely unexplored area of research based on their ability to resist ionizing radiation. Two approaches have been exploited to identify potentially valuable extremolytes and extremozymes (Madigan and Marrs, 1997). The traditional way requires scientists to grow at least small cultures of an extremophile obtained from a radiation hostile environment. In the other approach, the need to grow any cultures of extremophiles is bypassed and the DNA from all living things in a sample of water, soil or other material from an extreme environment is isolated. Recombinant DNA technology is then used to mine the genes for the enzymes from mixed populations of microbes without the need to culture extremophiles that might have trouble growing outside their native milieu (Madigan and Marrs, 1997). The molecules that enable radiation extremophiles to thrive are becoming valuable to the industries. As long as microbial prospectors can obtain sample genes from extremophiles in nature or from small laboratory cultures, they can generally clone those genes and use them to make the corresponding proteins (Madigan and Marrs, 1997).

Metabolic Products (Extremolytes and Extremozymes): Extremolytes are natural compounds which are synthesized by extremophilic microorganisms. Chemically, they are organic substances composed of amino acids, betain, sugar and heteroside derivatives (Lentzen *et al.,* 2006). Thus the name extremolyte is a coinage: organic substances (osmolytes) which are synthesized by extremophilic microorganisms. The presence of these molecules allows microorganisms to resist extreme living conditions like high radiation dose environment, drastic temperature variations and high salinity (Lentzen *et al.,* 2006, Graf *et al.,* 2008). Interestingly, these solutes are biologically inert and accumulate at high concentration in the cytoplasm without interfering with the overall cellular functions; hence they are called compatible solute.

Extremozymes are the enzymes produced by extremophiles for growth. Extremophiles are a potent source of extremozymes, which show outmost stability under extreme conditions (Adams *et al.,* 1995; Niehaus *et al.,* 1999; Demirjian, *et al.,* 2001; Fujiwara, 2002; Oren, 2002). Recent developments of extremozymes indicate that they are good novel catalysts of great industrial interest (Adams and Kelly, 1998). Typical examples are polymer-degrading enzymes like amylases, proteases, cellulases, pullulanases, and xylanases.

The application of extremophilic microorganisms in industrial processes has grown rapidly over the last two decades. Every category of these microbes has unique characteristics that can be harnessed for use in biotechnological industries (Adams and Kelly, 1998). Enzymes from these microbes, namely extremozymes, possess high stability and reduced risk of contamination of the organisms that produce them. Other useful features of these enzymes during the production process include improved transfer rates and lower viscosity. Due to the superior properties of these enzymes, they are expected to form the bridge between biological and chemical processes (Adams and Kelly, 1998). These enzymes have significant roles in chemical, food, pharmaceutical, paper, pulp, and waste-treatment industries (Adams *et al.,* 1995). In the pharmaceutical industry, membranes of some extremophiles have been found to contain surfactants bearing a unique stability. Other important innovative products are cyclodextrins, compatible solutes, and polyunsaturated fatty acids (Tango and Islam, 2000).

Ionizing Radiation, Biological Effects and its Mechanisms of Radiation Resistance: Unstable isotopes of certain elements decay spontaneously and change into another element within a biological system (Kiefer, 1990; Betlem *et al.,* 2012). During this process they emit ionizing radiation: radiation that is capable of ejecting electrons from their orbit around another atom, which charges the remaining particles and makes them ionized. This is dangerous, because it alters the chemical composition of molecules in cells of living organisms leading to mutations in the DNA, cancer and programmed cell death (apoptosis) if a repair does not take place (Kiefer, 1990; Betlem *et al.,* 2012).

Radioresistance (resistance against radioactivity) by extremophiles offers obviously two different actions: protection and repair. In the first case the cell tries to prevent the ionizing radiation

from doing direct and indirect damage to its vital molecules, for example by synthesizing antioxidants to counter the oxygen radicals. In the second case damage is not prevented, but the repair mechanisms are well-developed. This mends the DNA chains quickly and efficiently, making the chance that the fracture points are still close together as large as possible (Betlem *et al.,* 2012).
More so, resistance to ionizing radiation depends strongly on physiological conditions, such as the age of the culture, the cell concentration, the growth medium, the pH, the irradiation medium, the irradiation temperature, and the plating medium (Moseley *et al.,* 1972). The full recovery of an irradiated radioresistant extremophile like *Deinococcus radiodurans* for instance, is highly dependent on a rich source of nutrients, which are required for growth and the generation of Mn^{2+}complexes (Daly *et al.,* 2010; Ghosal *et al.,* 2005). Survival after facing life threatening from ionizing radiation exposure is affected greatly by the metabolic state of cells and the total dose delivered; 3kGy (50h at 60Gy/h) for *D. radiodurans* being lethal (Venkateswaran *et al.,* 2000). Cells grown in minimal medium and chronically irradiated with 60Gy/h rapidly lose viability coupled with severe DNA degradation, unless high concentrations of amino acids are provided (Venkateswaran *et al.,* 2000).

Radioresistant Organisms: Due to spontaneity connected with radioactive elements a number of high radioresistant species have evolved, all of which are able to survive huge amounts of radiation in comparison with humans. The following are some species of radioresistant microorganisms:

Chroococcidiopsis spp.: These cyanobacteria dominate the most extreme arid habitats in hot and cold deserts around the globe. Most of the time, they survive in a desiccated (frozen) state and are able to survive a dose of gamma radiation as high as 15kGy (Billi *et al.,* 2000).

Deinococcus radiodurans: Is one of the best documented radioresistant species. This bacterium is listed as the most resistant life form (as of May 2012) in the Guiness Book of Records. *D. radiodurans* is a red-pigmented, non-photosynthetic bacterium that is well known for its resistance to ionizing radiation (Anderson *et al.,* 1956; Daly and Minton, 1995; Cox and Battista, 2005; Slade *et al.,* 2009). This bacterium is exceptionally resistant to DNA-damaging agents, such as various forms of radiation, desiccation, miomycin C and hydrogen peroxide. Its high radioresistance mostly stems from the fact that *D. radiodurans* has a rapid DNA repair mechanism, coupled with a high level of highly effective antioxidants (Ji, 2010; Krinsky *et al.,* 2003).

Rubrobacter radiotolerans: This bacterium is relatively unknown, lives in Japan under radioactive conditions. It was first isolated from muddy water at a radioactive hot spring in Japan (Yoshinana, *et al.,* 1973). This pleomorphic rod-shaped, Gram-positive bacterium is highly radiotolerant, with a D_0 value of 10kGy, roughly 2.000 times the D_0 = 5Gy for humans (Rodriguez-Amaya, 2004). This extremely high radioresistance rests mainly on protection against radicals in the cell. It has high radioresistant properties; have two radio-protecting factors: superoxide dismutase and carotenoids (Terato *et al.,* 2011).

Thermococcus gammatolerans: This archaea species belongs to the order of *Thermococcales* and can withstand a dose of 3kGy without apparent lethality, with an exposure to higher doses only slightly reducing its viability (Tapias *et al.,* 2009).

Potential Activities of Radioresistant Extremophiles

Anticancer Drug Activity: Cancer is a multi-step disease (incorporating environmental, chemical, physical, metabolic, and genetic factors) which plays a direct or indirect role in the induction and deterioration of cancers (Fresco *et al.,* 2006). Strong and consistent evidence indicates that some extremolytes were anticipated to possess the ability to provide useful drugs, especially antibiotics and anticancer drugs, as well as agricultural products of commercial significance (Kumar *et al.,* 2010). Anticancer drugs are killers of cancer cells by arresting their growth at one or more checkpoints in the organism's cell cycle. Their main role is thus to reduce and prevent the growth and spread of cancer cells (Karnofsky, 1968).

The extremolytes are unique organic compounds that are not directly involved in normal growth, development or reproduction of organisms; however, their absence does affect the long-term impairment of the organism's survivability, ability to produce offspring, or appearance (Singh and Gabani, 2011).

Radioresistant extremophile with high consumption of antioxidant-rich metabolites significantly reduces the risk of many cancers, suggesting that certain extremolytes and/or extremozymes antioxidants could be effective agents for the prevention of cancer incidence and mortality (Singh and Gabani, 2011). These agents are a very promising group of compounds because they don't meddle with

the overall cellular functions of the organism and has outmost stability under extreme conditions (Adams *et al.,* 1995; Niehaus *et al.,* 1999). Consequently, in the last few years, the identification and development of such agents has become a major area of experimental research for the drug industry.
Extremolytic compounds constitute one of the most numerous and ubiquitous group of radioresistant metabolites, and are an integral part of the organism. It was found that in addition to their primary antioxidant activity, this group of compounds displays a wide variety of biological functions which are mainly related to modulation of carcinogenesis. Various *in vitro* and *in vivo* systems have been employed to determine the anticarcinogenic and anticancer potential of these natural compounds (Gabani and Singh, 2013).

For instance, the extremolyte bacterioruberin is a carotenoid resulting in red colour obtained from *Rubrobacter radiotolerans*. Its specific action in radioresistant microbes may have potential use in humans to repair damaged DNA strands caused by ionizing radiation such as ultraviolet radiation (UVR) and hence prevent skin cancer. More so, *Halobacterium salinarium*, another red-pigmented bacterium, containing bacterioruberin, is extremely resistant against UVR and hydrogen peroxide (H_2O_2) (Asgarani *et al.,* 2000). It was also documented that this organism is highly resistant to lethal actions of DNA-damaging agents including ionizing radiation and UV light, indicating a direct correlation between the presence of bacterioruberin and repair mechanisms involved in DNA repair (Shahmohammadi *et al.,* 1998).

Antioxidant Activity: Antioxidants are defined as compounds that can delay, inhibit, or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress. Oxidative stress is an imbalanced state where excessive quantities of reactive oxygen species (ROS, e.g., superoxide anion, hydrogen peroxide, hydroxyl radical) overcome endogenous antioxidant capacity, leading to oxidation of varieties of biomacromolecules, such as enzymes, proteins, DNA and lipids (Ames *et al.,* 1993). Oxidative stress is important in the development of chronic degenerative diseases including coronary heart disease, cancer and aging (Ames *et al.,* 1993). Antioxidant compounds are responsible for scavenging free radicals, which are produced during normal metabolism or during adverse conditions that can be harmful to biological systems and leading to death of an organism (Ames *et al.,* 1993).

Extremolytes like shinorine and porphyra-334 with origin from *Nodularia sp.* and *Microcoleus sp*. respectively showed a significant antioxidant drugs activity by inhibiting lipid peroxidation (de la Coba *et al.,* 2007; Singh *et al.,* 2008). The compound usurijene with organism origin from *Synechocystis sp.* also exhibited antioxidant activity against H_2O_2 (Zhang *et al.,* 2007), palythenic acid a compound isolated from *Maristentordinoferus* showcases also an invaluable therapeutic performance as an antioxidant (Lobban *et al.,* 2002). These compounds have led scientific support to the therapeutic usage of these extremolytes claimed in anticancer medicine at molecular biological level.

Biopterin and phlorotannin obtained from *Oscillatoria sp*. and *Ascophyllumnodosum* respectively (Li *et al.,* 2009; Mori *et al.,* 2010) when tested showed significant scavenging activity of intracellular ROS produced by $H_2O_{2;}$ suggesting their antioxidant properties. So it can be concluded that this isolated metabolic products are potent source for inhibiting the effects of ionizing radiation and may therefore be promising drugs for treatment of oxidative stress (Gabani and Singh, 2013). In present lifestyles where stress has taken an unwanted important position leading to excess production of free radicals these natural remedies will prove a support to our biological system to balance metabolism.
Another study that further support the antioxidant activity at molecular level are the extremolytes; sphaerophorin and pannarin obtained from lichens. Lichens are among the ultraviolet-tolerant organisms with secondary metabolites of pharmaceutical importance. These secondary metabolites have been considered in sunscreen protection ability for ultraviolet radiation (UVR) (Singh and Gabani, 2011). For instance, in the study carried out by Russo *et al.* (2008), the Chilean lichens were able to show protection against intense UVR. The effect of these extremolytes was evaluated on pBR322 DNA cleavage by hydroxyl radicals (-OH) and nitric oxide (NO-), and also for their superoxide anion (O_2^-) scavenging capacity.

Challenges and Prospects in Treatment: The isolation and maintenance of radiation-resistant microbes is a challenging task, as most extremophiles have specific nutritional requirements and growth conditions (Singh and Gabani, 2011). The fear of genomic instability, mutation prone habitat and scare of pathogenesis underscore their undesirable potential for proliferation in normal laboratory conditions. Limitations go beyond growth circumstances, as the extraction and purification of extremophiles have additional requirements. High-throughput screening methods are available to detect the microorganism's primary and secondary metabolites. This is by using advanced identification and study of gene sequences in the organisms DNA and the measurement of the metabolites of low molecular weight in the organism's cells at a specific time under specific environmental conditions (Singh 2006; Karsten *et al.,* 2009). However, once isolated from extremophiles, a chemical reference library devoted to extremolytes will be a significant tool for screening the therapeutic potential of novel microbial reserves.

Another challenging assignment is in the drug development companies, after years of thorough research to develop a specific drug. To observe the safety and efficacy at molecular level due to limited supply of transgenic animals for functional analysis of specific molecules derived from extremophiles is a problem. The isolation of purified extremolytes is among the limiting factors in developing these compounds for therapeutic usage (Singh and Gabani, 2011).

The frontier to the future of radiation extremophiles lies in the exploring of the mega-potential microorganisms found in extreme radiation environment. Radiation extremophiles has opened a new frontier of science. Biotechnology and bioremediation are major growth industries, providing the green technology of the future. Focusing on diverse bacteria that form the food web of the environment, these extraordinary microorganisms could provide solutions ranging from new medicines, to more effective bio-fuels and to new ways of treating contaminated wastewaters.

Conclusion

The application of radioresistant microorganisms to manufacturing and service industries and their use to produce products beneficial to man: using the identification and study of gene sequences in its DNA, the study of proteins expressed by genes within the organism, with applications in the understanding of disease and in drug development and the measurement of the metabolites of low molecular weight in the organism's cells at a specific time under specific environmental conditions is being highlighted. The goal of this overview has been to emphasize the latent possibilities of radioresistant microorganisms in the treatment of diseases or disorders especially cancer at molecular level. This aspect of knowledge has had little or no interest in this part of the world. It is envisaged that these processes may provide some insights into response of the microorganism's internal processes under different conditions. Researches on the metabolic products from radioresistant extremophiles have shown a sluggish pace in this part of the globe. The developmental process counts on the profitability base of the biotechnological industries and their curiosity for molecular level innovative concept from extremophiles. Recent advances in this concept are still lacking full authority; more studies will help in examining the unique properties of these radioresistant extremophiles and a reliable usage shall evolve.

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