Chapter 1: Review of literature

There is something fascinating about science. One gets such wholesale returns of conjecture out of such a trifling investment of fact. ~Mark Twain

Introduction

The ability of certain bacterial cells to resist ionising radiation is unusual due to apparent absence of niches that expose life forms to ionising radiation on Earth. Radiation resistance bacteria are represented in both the eubacteria and archaea, among which *Deinococcus*, *Rubrobacter* and *Kineococcus* represent genera whose members are largely radiation resistant. Most of the radiation-resistant bacteria reported are gram positive, with the exception of a radiation resistant gram-negative cyanobacterium, *Chroococcidiopsis* (Billi et al., 2000), gram positive species such as *Micrococcus luteus* (*Sarcina lutea*). *Methylobacterium radiotolerans* (Green and Brousfield, 1983), *Lactobacillus plantarum* (Hastings et al., 1983), *Acinetobacter radioresistens* (Nushimura and Izuka, 1988), *Enterococcus faecium* (van Gerwen, 1999), *Hymenobacter actinosclerus* (Collins et al., 2000), *Kocuria rosea* (Brooks and Murray, 1981) are some radiation resistant gram positive bacteria. Radiation resistance is widespread among hyperthermophilic archaea, for example, *Pyrococcus furiosus* (DiRuggiero, 1997), *Thermococcus gammatolerans* (Jolivet, 2003), and *Halobacterium* sp. (Kotemann, 2005). The scattered appearance of ionizing-radiation resistance among distinct prokaryotic lineages indicates two possibilities. First, radioresistance could be a vestige of DNA-repair mechanisms that were present in ancestral species and have been retained in those organisms that continue to require this phenotype. This explanation assumes that the ancestor's ability to cope with DNA damage has been lost by most descendents, and predicts that the molecular mechanisms of radioresistance should be similar among ionizing-radiation-resistant species. Second, given the infrequent occurrence of ionizing-radiation resistance, it is possible that this phenotype has arisen in unrelated species through horizontal gene transfer, or possibly convergent evolution (Cox and Battista, 2005).

The genus name — *Deinococcus* — was based on the Greek adjective 'deinos', which means strange or unusual; an apt description for an organism with an ability to survive excessive DNA damage that sets it apart from much of the life on Earth. Members of this genus are unique and are characterised by their ability to survive high doses of ionising radiation as well as non-ionising radiation.

1.1. Distribution and Phylogeny of *Deinococcus*

Deinococcus radiodurans R1 was the first radiation resistant bacterium isolated from spoiled canned meat (Anderson et.al., 1956). Till the beginning of this decade only seven members of this genus were reported while today the genus has more than 40 members and several 16S rDNA clone affiliations. Due to its pigmentation, gram positive nature initially *Deinooccus* were assigned to the genus *Micrococcus*. The family *Deinococcacae*, differentiated on the basis of morphology, consists of the *Deinococcus* and rod –shaped *Deinobacter*, represented by the only representative, *D. grandis*. On the basis of 16S rRNA gene sequence it was shown that deinococci formed a coherent group with the *Thermus* representing an ancient lineage in the Domain *Eubacteria* (Weisburg et al., 1989; Rainey, et al., 1997) (Fig. 1.1). Deinococci have been isolated from different niches from ordinary environment to stressed environments, a vast majority of which belong to desiccated environments. Table 1.1 lists the distribution of all the *Deinococcus* sp. that have been isolated till date with the source from which they were obtained (Slade and Radman, 2011).

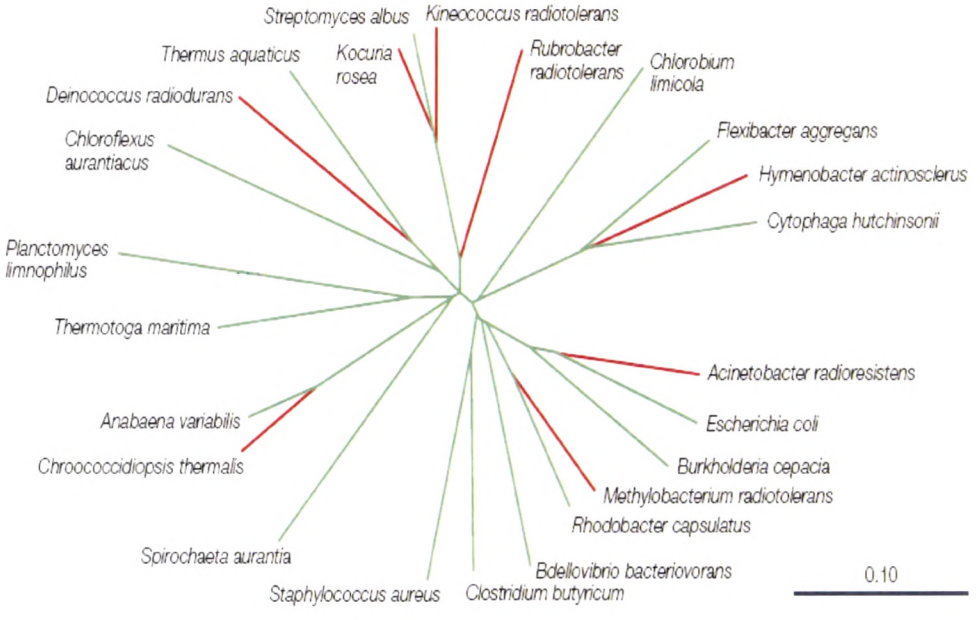

Fig. 1.1: Phylogenetic distribution of radiation–resistant *Eubacteria* (Cox and Battista, 2005). The red line depicts radiation resistant taxa.

Table 1.1: Source of type strains of *Deinococcus* species isolated till date.

S. No.	*Deinococcus* species	Source	Reference
1.	*D. radiodurans* DSM 20539^T	Gamma-irradiated canned meat	Anderson et al., 1956
2.	*D. radiopugnans* ATCC19172^T	Haddock tissue	Davis et al., 1963
3.	*D. radiophilus* DSM 20551T	Mumbai duck	Lewis et al., 1971
4.	*D. proteolyticus* DSM 20540^T	Feces of a llama	Kobatake et al., 1973
5.	*D. grandis* DSM 3963^T	Feces of an elephant	Oyaizu et al., 1987
6.	*D. geothermalis* AG-3a^T	Hot spring	Ferreira et al., 1997
7.	*D. murrayi* ALT-1b^T	Hot springs	Ferreira et al., 1997
8.	*D. indicus* Wt/1a^T	Groundwater	Suresh et al., 2004
9.	*D. frigens* AA692^T	Antarctic soil	Hirsch et al., 2004
10.	*D. saxicola* AA1444^T	Antarctic sandstone	Hirsch et al., 2004
11.	*D. marmoris* AA63^T	Antarctic marble	Hirsch et al., 2004
12.	*D. hohokamensis* KR-40^T	Sonoran desert soil	Rainey et al., 2005
13.	*D. navajonensis*KR-114^T	Sonoran desert soil	Rainey et al., 2005
14.	*D. hopiensis* KR-140^T	Sonoran desert soil	Rainey et al., 2005
15.	*D. apachensis*KR-36^T	Sonoran desert soil	Rainey et al., 2005
16.	*D. maricopensis* LB-34^T	Sonoran desert soil	Rainey et al., 2005
17.	*D. pimensis* KR-235^T	Sonoran desert soil	Rainey et al., 2005
18.	*D. yavapaiensis* KR-236	Sonoran desert soil	Rainey et al., 2005
19.	*D. papagonensis*KR-241^T	Sonoran desert soil	Rainey et al., 2005
20.	*D. sonorensis* KR-87^T	Sonoran desert soil	Rainey et al., 2005
21.	*D. deserti* VCD115^T	Sahara desert sand	de Groot et al., 2005

22.	*D. ficus* CC-FR-10 ^{T}	Rhizosphere of *Ficus religiosa*	Lai et al., 2006
23.	*D. mumbaiensis* Con-1 ^{T}	Contaminated agar plate	Shashidhar and Bandekar, 2006
24.	*D. peraridilitoris* KR-200 ^{T}	Coastal desert	Rainey et al., 2007
25.	*D. radiomollis* PO-04-20-132 ^{T}	Alpine environments	Callegan et al., 2008
26.	*D. claudionis* PO-04-19-125 ^{T}	Alpine environment	Callegan et al., 2008
27.	*D. altitudinis* ME-04-32 ^{T}	Alpine environment	Callegan et al., 2008
28.	*D. alpinitundrae* ME-04-04-52 ^{T}	Alpine environment	Callegan et al., 2008
29.	*D. aquaticus* PB 314 ^{T}	Freshwater	Im et al., 2008
30.	*D. caeni*Ho-08 ^{T}	Activated sludge	Im et al., 2008
31.	*D. aquatilis* CCUG 53370 ^{T}	Water	Kampfer et al., 2008
32.	*D. aquiradiocola* TDMA- uv53 ^{T}	Radioactive site	Asker et al., 2009
33.	*D. xinjiangensis* X-82 ^{T}	Desert soil	Peng et al., 2009
34.	*D. gobiensis* I-O ^{T}	Gobi desert	Yuan et al., 2009
35.	*D. aerius* TR-0125 ^{T}	High atmosphere	Yang et al., 2009
36.	*D. piscis* 3ax ^{T}	Marine fish	Shashidahr and Bandekar, 2009
37.	*D. aetherius* DSM 21230 ^{T}	Stratosphere	Yang et al., 2010
38.	*D. aerolatus* JCM 15422 ^{T}	Air	Yoo et al., 2009
39.	*D. aerophilus* JCM 15443 ^{T}	Air	Yoo et al., 2009
40.	*D. wulumuquiensis* NBRC 105665 ^{T}	Radiation-polluted soil	Wang et al., 2009
41.	*D. xibeiensis* NBRC 105666 ^{T}	Radiation-polluted soil	Wang et al., 2009
42.	*D. guangriensis* JCM 15082 ^{T}	Radiation Centre	Sun et al., 2009
43.	*D. depolymerans* TDMA-24 ^{T}	Radioactive freshwater site	Asker et al., 2010

1 .2 General features of *Deinococcus radiodurans*

Deinococcus radiodurans R1 has been the major model of study for radiation resistance. The genome of *D. radiodurans* R1 (ATCC BAA-816) has been sequenced. The *D. radiodurans* chromosome is 3.28 Mb, with a GC content of 66.6%. The genome is segmented and consists of a 2.64 Mb chromosome (chromosome I), a 0.41 Mb chromosome (chromosome II), a 0.18 Mb megaplasmid and a 0.045-Mb plasmid (White et al., 1999). The members of the genus *Deinococcus* are exceptionally resistance to radition ionising (X rays and γ rays) as well as non-ionising radiation (ultravoilet, UV), oxidising agents as H_2O_2 ($OH^•$ generator) and paraquat ($O_2^•$ generator) as well as several mitogenic agents as mitomycin C (MMC).

There are two types of ionizing radiation, both produced by the decay of radioactive elements: electromagnetic (X and gamma radiation) and particulate (α and β particles) (Cox and Battista, 2005). Gamma rays are photons that generate ions, which react with other molecules to produce free radicals. Reaction with water molecules gives rise to hydroxyl radicals ($OH^•$), the most reactive oxygen species (ROS) (Imaly, 2003; Ghosal et al., 2005). Fig. 1.2a depicts the ionisation effects of the different forms of ionising radiation. Ionizing radiation (IR) generates multiple types of DNA damage: base damage, SSBs, DSBs, and interstrand cross-links (Fig. 1.2b). DNA bases are most affected, with more than 80 different types of structural modifications induced by ionizing radiation. Approximately 10% to 20% of the time, the sugar-phosphate moiety is affected, which can lead to a single-strand break (Bjellard and Seeberg, 2003). On average, for every 20 SSBs induced by gamma rays in DNA, there is 1 DSB (Slade and Radman, 2011). If not repaired, DSBs prevent the replication of genomes and lead to cell death. Radiation-resistant and radiation-sensitive species have remarkably similar numbers of DSBs per Gy per genome (0.002 to 0.006 DSBs/Gy/Mbp) (Gerard et al., 2001; Rothkamm and Lobrich, 2003) but differ in the amounts of oxidative DNA base damage (Kish et al., 2009). Fig. 1.2c shows that *D. radiodurans* can endure approximately 160 DSBs/ haploid genome without any mutation frequency whereas the radiation sensitive organism like *E. coli* shows 90% killing with about 6 DSB in its genome. Table 1.2 lists the D_{10} vlue for radiation resistant strains and DSBs caused by IR in the organisms listed.

D. radiodurans is extremely resistant to UV-C radiation (100 to 295 nm) and can efficiently repair UV-induced bipyrimidine photoproducts (BPPs), cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts (6-4 PPs) (Blasius et al., 2008). The major BPP in UV-irradiated *D. radiodurans* is CPD TC (47.2%), while 6-4 PPs are least represented (Moeller et al., 2010). Following 500 J/m^2 of radiation, more than 80 % of thymine-derived photoproducts are removed from *D. radiodurans* cells within 90 min (Blasius et al., 2008; Cox and Battista, 2005) and appear in the form of di- and trinucleotides in the medium outside the cells (Battista, 2005).

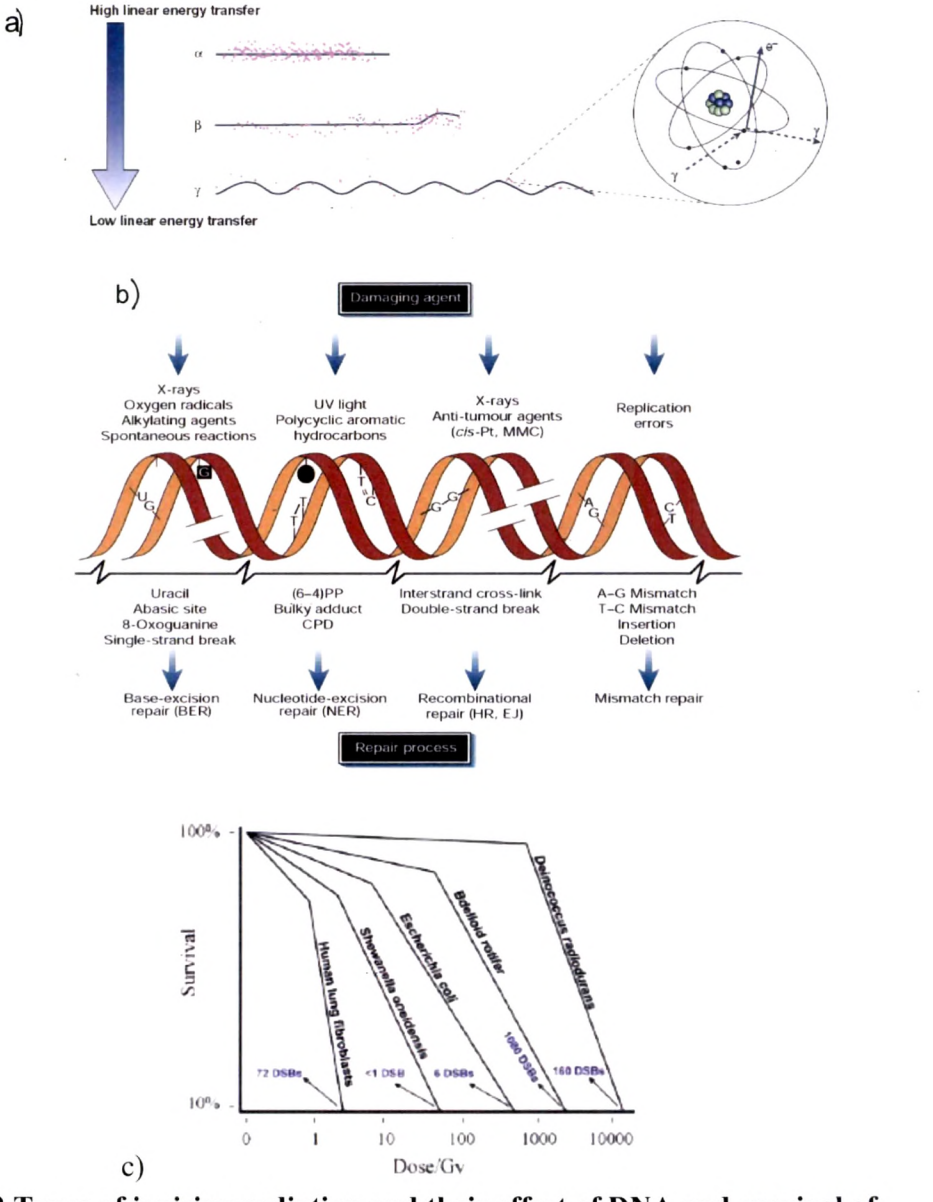

Fig. 1.2 Types of ionising radiation and their effect of DNA and survival of organisms. a) The tracks of three different types of ionizing radiation. Small dots

indicate energy deposition events(Cox and Battista, 2005); b) Types of DNA damage bydifferent DNA damaging agents, including radiation, and repair processes involved; c) Survival curves of representative organisms exposed to γ radiation. DSBs inflicted per haploid genome at D_{10} are indiacted by arrows Daly, 2011).

Table 1.2 Compartive account of radiation resistance in bacteria

Strain	Genome size[a]	D_{10} (kGy)	DSB/Gy/Mbp (approximate linear density of DSBs in vivo)	Mn/Fe ratio	Reference
Deinococcus radiodurans	3.28	16	0.003	0.24	Ghosal et al., 2005
D. geeothermalis	3.23	10	ND	0.46	Ghosal et al., 2005
Truepera radiovictrix	3.26	5.0[b]	ND	ND	Alberqueque et al., 2005
Kineococcus radiotolerans	4.76	2.0	ND	0.087	Bagwell et al., 2008
Enterococcus faecium		2.0	ND	0.17	Daly et al., 2004
Escherichia coli	4.64	0.7	0.006	0.0072	Ghosal et al., 2005
Pseudomonas putida	6.18	0.25	ND	<0.0001	Ghosal et al., 2005
Shewnella onedensisi	5.13	0.07	0.002	0.0005	Ghosal et al., 2005
Archeal isolates					
Halobacterium salinarum	5.2	5.0	0.002	0.19	Robinson et al., 2011
Thermococcus radiotolerans	2.05	8	ND	ND	Jolivet et al., 2004
Pyrococcus furiousus	1.91	2.5[c]	0.007	ND	Gerard et al., 2001

[a] *http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db = genomeprj & cmd = Retrieve & dopt = Overview & list_uids = 65.*

[b] D_{60} dose that causes 40 % killing.

[c] D_{75} dose that cuases 25 % killing.

1.2.1 Physical structure of Deinococcus radiodurans

Although *D. radiodurans* is gram positive, the cell envelope is reminiscent of gram-negative bacteria due to its multilayered structure and lipid composition. The cell envelope of *D. radiodurans* is unusual in terms of its structure and composition.

At least six layers have been identified by electron microscopy, with the innermost layer being the plasma membrane. A few strains of *Deinococcus* also exhibit a dense carbohydrate coat. Only the cytoplasmic membrane and the peptidoglycan layer are involved in septum formation during cell division. Fig. 1.3b shows the distribution of the layers of deinococcal cell wall (Rofthussus et al., 2006). The diamino acid L-ornithine found in the mucopeptide is the signature amino acid of the genus *Deinococcus*s (Murray, 1986).

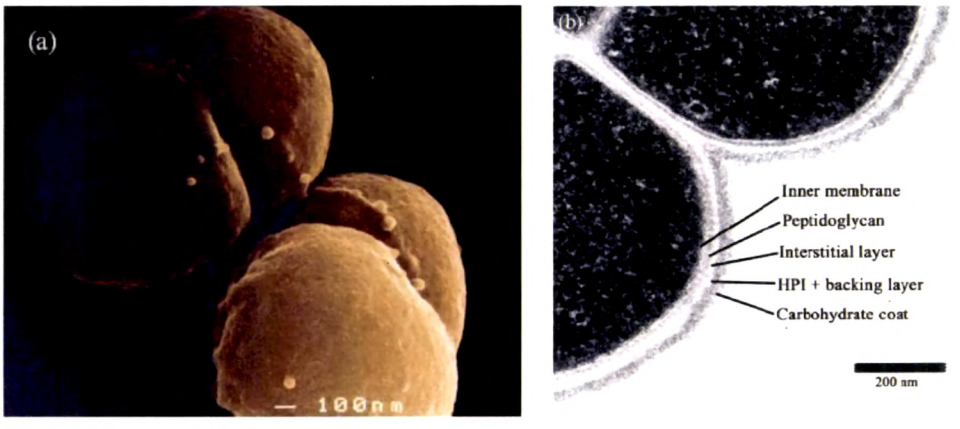

Fig. 1.3 Scanning electron micrograph of *Deinococcus radiodurans* R1 a) cells; b) cell wall (Rothfuss et al., 2006).

1.2.3 Metabolic configuration of *Deinococcus radiodurans*

D. radiodurans is an organotrophic bacterium with a proteolytic life-style (Ghosal et al., 2005). Amino acids are a preferred primary carbon energy source (He, 2009; Zhang et al., 2000), while carbohydrates are preferred in the following order: fructose > pyruvate > lactate >glucose> oxaloacetate > glycerol (Venkateswaran et al., 2000). *D. radiodurans* is dependent on exogenous nicotinic acid because it lacks key enzymes for NAD biosynthesis (Holland et al., 2006). Its methionine auxotrophy can be alleviated with vitamin B12, which is required as a cofactor for methionine synthase (Holland et al., 2006). In the presence of vitamin B12, sulfate can be used as the sole sulfur source (Holland et al., 2006).

Several metabolic properties help *D. radiodurans* to surmount oxidative stress: (i) proteolysis and the import of exogenous peptides and amino acids(Zhang et al,2000; Ghosal et al, 2005;), (ii) the conversion of glucose via the Pentose phosphate Pathway (PPP) into precursors for deoxynucleoside triphosphates (dNTPs) (Zahng et al.,2003), (iii) the suppression of ROS production by the induction of the glyoxylate bypass of the tricarboxylic acid (TCA) cycle and a reduction in the number of

respiratory chain enzymes and enzymes with iron-sulfur clusters (Daly et al., 2010; Makarova et al., 2007), (iv) metabolic defects resulting in metabolite accumulation, and (v) carbohydrate and polyphosphate storage granules(Daly et al., 2010).

Interestingly, *D. radiodurans* R1 has been shown to have absolute requirement for Mn^{2+} to support normal growth in minimal medium (Daly et al., 2004). It has been shown to accumulate Mn^{2+} as reflected in higher Mn/Fe ratio as compared to the radition sensitive bacterial strains (Table 1.2) (Daly et al., 2004; Ghosal et al., 2005). The addition of Mn^{2+} to the stationary phase culture of *D. radiodurans* R1 has been demonstrated to initiate fresh rounds of replication (Chou and Tan, 1990). Ammendement of Mn^{2+} to the mediun shifts the mode of glucose metabolism from PPP to TCA, making the cells sensitive to UV radiation (Zhang et al., 2000; Zhang et al., 2003).

1.3 Cellular damage caused by radiation

The central dogma of radiation biology is that the cytotoxic and mutagenic effects of radiation are the result of DNA damage principally by indirect effects mediated by HO• (Ghosal et al., 2005; Daly et al., 2007). Water is the most abundant chemical found in living cells and the primary ROS which arise during the radiolysis of H_2O are HO•, $O_2^{\bullet-}$

$$H_2O \xrightarrow{\text{Radiolysis}} OH\bullet + H^+ + e^-_{aq}$$

$$2\,OH\bullet \longrightarrow H_2O_2$$

$$O_2 + 2e^-_{aq} \longrightarrow O_2^{\bullet-}$$

Oxygen species are small molecules that cannot easily be excluded from active sites, and if they contact redox cofactors at a lower potential than themselves, then electron transfer can occur.

$$O_2 \xrightarrow[-0.16\ V]{e^-} O_2^- \xrightarrow[+0.94\ V]{e^-,\,2H^+} H_2O_2 \xrightarrow[+0.38\ V]{e^-,\,2H^+} HO\bullet \xrightarrow[+2.33\ V]{e^-,\,H^+} OH^-$$

Reactions of this type are responsible both for the formation of ROS, that majorly consists of O^{2-} and the $HO^{\bullet}$, also for their subsequent inactivation of enzymes. The only oxygen species that can directly damage most biomolecules is $HO^{\bullet}$. Several transition metals such as Fe, Cu, Cr, V can directly catalyse the Fenton type chemistry to produce the reactive $HO^{\bullet}$, while other metals as Cd, Hg, Pb are known to produce ROS albeit indirectly by either replacing essential metals from their active centres as in case of Cd or depletion of the sulfhydryl group that reduces the

reducing power of the cell. (Stohs and Bagchi, 1995). The hydroxyl radical oxidizes most organic molecules at diffusion-limited rates. While the Fenton reaction has been linked to protein carbonylation and membrane peroxidation, its most significant impact is likely to be upon DNA, since even a single DNA lesion is potentially mutagenic or lethal (Imlay, 2003; Imlay, 2008).

The fact that DSBs caused by radiation are essentially the same in bacterial genomes indicates that the target of radiation imposed damage is not limited to just DNA and other bio molecules are equally vulnerable targets of the radiation inflicted damage. Recently, fresh insight into the reparability of DSBs was gained by comparisons of DNA and protein damage in irradiated bacteria which have very different antioxidant levels and resistances. For a given dose of ionizing radiation, DSB lesion-yields were very similar, but protein oxidation lesion-yields were quantitatively related to survival (Daly et al., 2007; Daly, 2010; Krisko and Radman, 2010). .

1.3.1 Protein carbonylation (PC)

Carbonylation is the most common oxidative modification of proteins, often used as a biomarker of oxidative stress and has been demonstrated to be the cause of the radiation induced damage to the cell. PC content in irradiated and unirradiated cells of DR1 is lower than those determined for radition sensitive organisms (Daly et al., 2007). The accumulation of oxidative damage to proteins alters their catalytic activities and interactions, which leads to the disruption of cellular functions and culminates in cell death (Nystrom, 2005; Slade and Radman, 2011). The oxidation of DNA repair proteins causes error-prone activities, which result in DNA mutations (Daly et al., 2007).

Carbonyl derivatives are formed by a direct metal catalyzed oxidative (MCO) attack on the amino-acid side chains of proline, arginine, lysine, and threonine. In addition, carbonyl derivatives on lysine, cysteine, and histidine can be formed by secondary reactions with reactive carbonyl compounds on carbohydrates (glycoxidation products), lipids, and advanced glycation/lipoxidation end products. The quantitatively most important products of the carbonylation reaction are glutamic semialdehyde from arginine (Fig. 1.4) and proline, and aminoadipic semialdehyde from lysine. Compared to other oxidative modifications, carbonyls are relatively difficult to induce and in contrast to cysteine disulfide bond formation,

carbonylation is an irreversible oxidative process. Thus, a cell must rid itself of carbonylated proteins by degrading them (Nystrom, 2005). Carbonylation of proteins may occur during an increased ROS production, diminished ROS defence, or reduced protease activity (Frederickson et al., 2004; Avery, 2011).

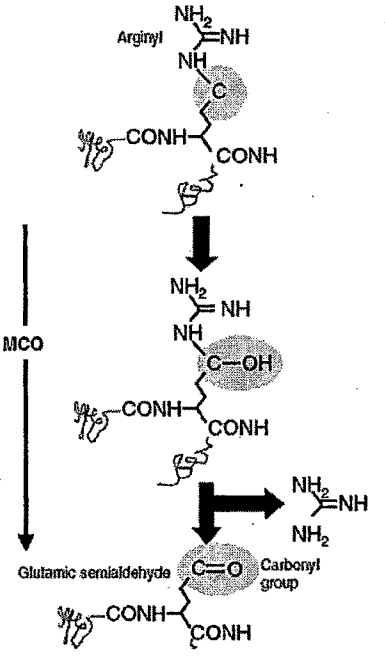

Fig. 1.4 Carbonylation process in protein (Nystrom, 2005).

1.3.2 Membrane damage

Biological membrane serves as impermeable barriers and function in cellular transport processes, therefore severe membrane dysfunction is usually associated with loss of viability. Among the ROS, the protonated form of the superoxide anion and the hydroxy radical commonly initiate the process of autocatalytic lipid peroxidation (Imlay, 2003). Transition metals also catalyse lipid peroxidation. It is likely that a lipid peroxidation chain reaction begins after hydrogen abstraction from an unsaturated fatty acid to form a lipid radical. The lipid radical (L) thus formed reacts with molecular oxygen to form a lipid peroxy radical ($ROO^{\cdot}$). The reaction is perpetuated when the lipid peroxy radical attacks another unsaturated fatty acid and abstracts a hydrogen atom to form a fatty acid hydroperoxide (ROOH) and perpetuate the initial reaction. The hydroperoxides thus formed will break down thermally or in the presence of O_2 or reduced transition metals to form lipid peroxy radicals ($LOO^{\cdot}$) or lipid alkoxy radicals ($LO^{\cdot}$), both of which can initiate new rounds of peroxidation (Farr and Kogoma, 1991).

Lipid alkoxy radicals can undergo cleavage of C-C bonds to form unsaturated fatty acid aldehydes and alkyl radicals. In addition to producing fatty acyl chains that are shorter than the parent chain, the end products of lipid peroxidation include alkanes, ketones, epoxides, and aldehydes. The net result of lipid peroxidation is conversion of unsaturated lipids into polar lipid hydroperoxides, which can cause increased membrane fluidity, efflux of cytosolic solutes and loss of membrane protein activities. Extensive lipid peroxidation has been correlated with the ultimate disintegration of membrane integrity and cell death, but it has rarely been resolved whether it is a cause or effect of death. The rate of fatty acid peroxidation is directly proportional to the number of unsaturated C=C bonds. Transition metals have been reported to cause lipid peroxidation in bacteria (Avery, 2011).

1.3.3 DNA damage

Cellular exposure ionizing radiation results in numerous types of DNA lesions. In addition to the DNA damage caused directly by oxygen radicals, intermediate organic radicals that are formed during the propagation step of lipid peroxidation can react with DNA causing trand breaks. Strand breaks and other lesions that block replication are likely to contribute more toward lethality than base damage that does not hinder replication, although the latter may contribute significantly to mutagenesis (Farr and Kogoma, 1991). Simultaneous inactivation of functions involved in BER(Base Excision Rep and NER (nucleotide excision repair) yield strains that are sensitive to lethal mutagens, presumably via oxidative DNA lesions (Kuzmin et.al, 2005). At the same time, DNA repair-related mutants are ROS sensitive, linking this lethality to oxidative DNA damage including gross chromosomal rearrangements and instability (Avery, 2011; Imlay and Linn, 1988; Hasset and Cohen, 1989). In *E. coli*, DNA may be a more important ROS target in organisms where membrane lipid oxidation is less likely (Avery, 2011). In cases where lethal DNA damage is linked to pro-oxidant toxicity, the primary target can in fact be protein(s) required for preserving DNA integrity. Here, elevated DNA damage is a secondary outcome of direct protein inactivation (Imlay and Linn, 1988). Finally, DNA damage itself can result in elevated ROS eneration, with the potential to attack other targets which may be more pivotal for cell viability (Avery, 2011).

1.4 Radiation Resistance mechanism in *Deinococcus radiodurans* R1

The remarkable capacity of *D. radiodurans* R1 to withstand ionising and non-ionising form radiation has attracted the major deinococcal research. It can also withstand several DNA mutagenic agents the repair mechanism of which overlaps the mechanism involved in radiation resistance.

1.4.1 Resistance to UV-C Radiation

D. radiodurans possesses the classical nucleotide excision repair pathway (UvrABC) for the removal of pyrimidine dimers. It involves a protein complex (UvrABC excinuclease) that recognizes the structural changes in DNA caused by UV damage and creates the dual incisions 5′and 3′ to the damaged site. The UV damage endonuclease (UVDE) pathway (Minton, 1994; Mosley and Evans, 1983) is mediated by endonuclease (*uvsE*), which has a novel requirement for manganese ions and an endonucleolytic mode of action that is different from that of UvrABC (Evans and Mosley, 1983; Mosley and Evans, 1983).

The two pathways have overlapping functions, as both need to be inactivated to produce a UV sensitive phenotype (Mosley and Evans, 1983). A *uvrA uvsE* double mutant is 100 fold more sensitive to 250 J/m^2 UV than the wild type and loses the shoulder of UV resistance (Earl et al., 2002). The slightly higher UV sensitivity of the *uvrA* mutant than the *uvsE* mutant suggests thatUvrABC is more important for UV resistance than is UVDE (Slade and Radman, 2011). In addition, UvrABC is constitutively expressed which indicates that UvrABC is important for the continuous removal of damaged nucleotides from the cells (Lipton et al., 2002). Both the UvrABC and UVDE pathways require Pol I, as the *polA* mutant is equally sensitive to UV radiation as the *uvrA uvsE* double mutant (Gutman et al., 1993).

The recombination-deficient *recA* mutant is more sensitive to UV radiation than is the *uvrA uvsE* mutant, which suggests that recombinational repair is more significant than the two excision repair pathways for UV radiation resistance in *D. radiodurans* (Tanaka et al., 2005). Mutations in other recombination genes, *recO* and *recF*, also result in UV sensitive phenotypes (Chang et al., 2010; Xu et al., 2008). Unlike ionizing radiation, UV does not induce point mutations in *D. radiodurans*, even at doses as high as 1,485 J/m^2 (Tanaka et al., 2005). The absence of translesion synthesis (TLS) DNA polymerases in *D. radiodurans* (Makarova et al., 2001) contributes to the high fidelity of the repair of UV lesions.

1.4.2 Resistance to Ionising Radiation

D. radiodurans R1 can sustain gamma irradiation doses that introduce hundreds of double-strand breaks in its genome. The kinetics of DNA double-strand break repair is very rapid as an intact genome complement is reconstructed from a myriad of fragments in few hours (Fig. 1.5a) (Blasius et al., 2008).

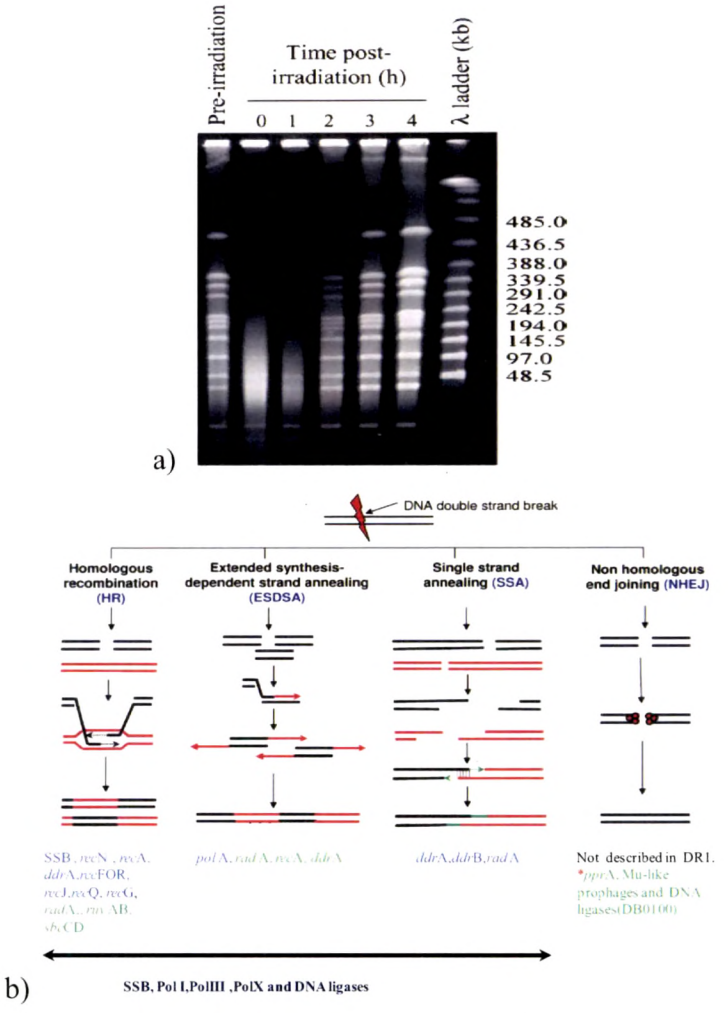

Fig. 1.5 Kinetics and repair of DSB in D. radiodurans a) Kinetics of DNA DSBs in *D. radiodurans* cells post-γ –irradiation and analysed by PFGE; b) Different pathways for DSB in D. radiodurans R1. Genes involved in each of the pathways are indicated in blue while of the proposed function but not expremintally demonstrated are shown in green. Common proteins involved in DSB repair is indicated below the arrow (Blasius et al., 2008).

Several mechanisms have been proposed to account for such an efficient repair (Figure 1.5b). Homologous recombination (HR) in *D. radiodurans* R1 forms a bulk of repair post-irradiation and involves essentially the same proteins that are

employed in *E.coli* recombitional repair. In *D. radiodurans* R1 HR can be divided into Rec A dependent and Rec A independent pathway recovery. Major proteins involved in the recombinational repair in *D. radiodurans* R1 are listed in Table 1.3.

Table 1.3: Role of major proteins involved in recombinational repair in *D. radiodurans* R1 (compiled from Slade and Radman, 2011)

Gene	Function	Phenotype of the mutant	Reference
rec J (DR1226)	5′–3′ single- strand-specific exonuclease activity, producing 3′ ends	Modestly sensitive to gamma rays and only slightly sensitive to UV and H_2O_2	Bentchikou et al., 2010 Cao et al., 2010
*uvr*D (DR1775)	Major helicase	Moderately sensitive toγ radiation. Delayed DNA synthesis and reassembly.	Bentchikou et al., 2010
recQ (DR1289)	Helicase unwinds DNA 3′–5′	No effect on radiation resistance but highly sensitive to MMC, UV and H_2O_2	Bentchikou et al., 2010 Huang et al., 2007
*rec*N (DR1477)	Tethers DNA molecule in a cohesion-like fashion and prevents the separation of DNA	Sightly increased sensitivity to gamma rays, UV radiation, and MMC	Funayama, T., et al., 1999
rec FOR *rec*F (DR1089) *rec*O (DR0819) *rec*R (DR0198)	loads RecA onto the 3′-tailed DNA coated with SSB'	Extremely sensitive to gamma rays, and display incomplete genome reconstitution,a reduced level of DNA breakdown, and absence of DNA synthesis	Xu et al., 2008. Bentchikou et al., 2010
*rec*A (DR 2340)	Homologous recombination	Reduced γ, UV and MMC resistance	Slade and Radman, 2011
rad A (DR1105)	assist RecA in priming DNA repair synthesis during ESDSA,	moderately sensitive to ionizing radiation and have a delay in repairing	Slade et al., 2009, Zhou et al., 2006
*ruv*A (DR1274), *ruv*B (DR0596)	RuvAB complex stimulates the branch migration of Holliday junctions in the 5′-to-3′	ND modestly sensitive to UV radiation, gamma rays, and	Tsaneva et al., 1993 Kityama et al.,

	direction	MMC	1997
rec G (DR1916)	branch migration of Holliday junctions in 3′-to-5′ direction,	highly sensitive to gamma rays and H_2O_2	Whitby et al., 1993.
*rec*D (DR1902)	helicase activity with 5′ -3′ polarity with low processivity	enhances the efficiency of transformation by exogenous homologous DNA and has anti- recombigenic properties.	Shadrick and Julin , 2010
	Novel *Deinococcus* repair protein		
ppr A (DRA0346)	stimulates DNA end-joining reactions catalyzed by ATP- and NAD-dependent DNA ligases	highly sensitive to ionizing radiation,MMC,andUV-A radiation	Narumi et al., 2004; Bauermeister, 2009
DdrA (DR0423)	protects 3′ ssDNA overhangs from degradation by *E. coli* exonuclease		Harris et al., 2004; Omelchenko et al., 2005

1.4.2.a. RecA dependent pathway

The recombinational repair of double strand breaks (DSBs) in *D. radiodurans* proceeds via two homologous recombination processes, extended synthesis dependent starnd annealing (ESDSA) and homologous recombination by crossovers, both of which rely on the RecA recombinase (Blasius et al., 2008; Cox and Battista, 2005). RecA and its homolog, RadA, prime DNA repair synthesis on partially overlapping fragments as templates (Slade et al., 2009; Zahradka, 2006)). RecA is essential, as RadA cannot replace RecA-mediated DNA synthesis priming. Following RecA-RadA-catalyzed priming, DNA Pol III initiates DNA repair synthesis (Slade et al., 2009). DNA repair synthesis generates long newly synthesized single strands, which processively dissociate from the migrating D loops, aided by DNA helicases, and can readily anneal with complementary strands. The 3′ flaps generated after the annealing of single strands could be incised by SbcCD. The long linear products of ESDSA require RecA-mediated crossovers within overlapping homologies to mature into circular chromosomes (Zahradka, 2006).

1.4.2.b. Rec A independent pathway

In the absence of RecA, approximately one-third of the DSBs generated by ionizing radiation can be rejoined by a RecA-independent pathway (Slade and Radman, 2009; Zahradka et al., 2006). The RecA-independent single strand annealing (SSA) pathway may involve proteins such as DdrA, which protects 3′ ssDNA ends from degradation (Harris et al., 2004); DdrB, an SSB-like protein with strand-annealing properties (Norais et al., 2009; Sheng et al., 2005); and RadA, a distant RecA homolog (Slade et al., 2009). The lesser extent of DNA degradation observed for the *recA* mutant (Slade et al., 2009) is congruent with the importance of protecting the DNA fragments' ends in the absence of RecA before annealing with overlapping fragments can occur. In the absence of RecA, RadA also seems to contribute to the RecA independent pathway of DSB repair, although its role remains unclear (Slade et al., 2009).

1.5 Models of radiation resistance in *D. radiodurans* R1

Although DNA repair proteins in *D. radiodurans* R1 are enzymatically very similar to those in other bacteria, their remarkable efficiency in assembling DNA fragments may be partially imparted by other features of the organism. Of the several hypothesis that have been forwarded for the radiation resistance in *D. radiodurans* R1 key hypothesis are as follows.

1.5.1 Chromosome alignment and nucleoid morphology facilitate genome reassembly

Several models explain how structural aspects may contribute to the observed rapidity and efficiency of the RecA mediated homology search in *D. radiodurans* R1: (i) genome condensation, (ii) ring-like nucleoid morphology, (iii) DNA-membrane association, and (iv) chromosome alignment. Absolute role of any one single physical attribute has not been established. This model made two major predictions: first, *recA*-dependent recombination between homologous DSB fragments originating from widely separated genomic locations should show strong positional effects on irradiation and, second, transmission electron microscopy (TEM) of chromosomal DNA in *D. radiodurans* should reveal evidence of structures linking chromosomes. Both predictions were tested and refuted: molecular studies showed high levels of recombination between homologous DSB fragments irrespective of their genomic origin (Daly et al., 1994; Daly and Minton, 1995, 1996); and no linking structures were observed by TEM-based optical mapping (Lin

et al., 1999). Another model proposed that high levels of chromosomal condensation observed in *D. radiodurans* grown in rich medium facilitated repair by holding proximal DSB ends together and that manganese promoted the condensation of its nucleoids into ringlike structures (Levin-Zaidman et al., 2003). This model is also generally discounted: *D. radiodurans* grown in defined minimal medium (DMM) did not display condensed nucleoids but remained extremely IR resistant and *D. radiodurans* that was depleted in manganese displayed condensed ring like nucleoids but was rendered IR sensitive (Daly et al., 2004; Ghosal et al., 2005). Thus, IR-induced DSB fragments in irradiated *D. radiodurans* are not immobilized and the structural form of its nucleoids does not play an important role in radioresistance

1.5.2 Subset of uncharacterized genes encode novel proteins that enhance the efficiency of DNA repair

Experimental evidence supporting that *D. radiodurans* relies, at least in part, on a core set of ordinary DNA repair proteins is now well established (Blasius et al., 2008; Cox and Battista, 2005; Makarova et al., 2007; Slade et al., 2009). Whole transcriptome studies on irradiated *D. radiodurans* were used to identify novel genes induced during recovery (Liu et al., 2003; Tanaka et al., 2004); there are only approximately 150 uncharacterized genes that are shared between the three *Deinococcus* genomes. Among those which were induced in irradiated *D. radiodurans*, only few have a discernible functional relevance to the preservation of genome integrity. Another moderately IR-sensitive *D. radiodurans* mutant is *pprA2*, which is a putative DNA-binding protein (Kota and Misra, 2006). However, for most of the mutants derived from this subset of novel genes, there was no drastic change in the level of IR resistance, indicating that few of the putative resistance proteins, at least individually, make a substantial contribution to the recovery of irradiated *D. radiodurans*. Thus, functional genomics evidence supporting this hypothesis has grown progressively weaker (Makarova et al., 2007).

1.5.3 Manganese as protective agent against IR

Hydroxyl radicals are the primary reactive oxygen species (ROS) generated by IR and indiscriminately damage all macromolecules (Imlay, 2003). It has been proposed that naturally sensitive bacteria are killed by IR mainly owing to protein oxidation, whereas manganese complexes in extremely resistant bacteria protect enzymes needed to repair DNA and allow survival (Daly, 2010). This observation correlated

well with the intracellular Mn/Fe ratio in the radiation resistant cultures. The role of accumulated manganese in the chemical removal of ROS has been ascribed to the formation of small complexes. Inorganic phosphate and Mn^{2+} form complexes that catalytically remove superoxide (Barnese et al., 2008) and amino acids and peptides form complexes with Mn^{2+} that catalytically decompose hydrogen peroxide (Berlett et al., 1990). The formation of Mn^{2+} complexes is highly dependent on the availability of inorganic phosphate and free amino acids or peptides and other small molecules. Thus, the strong trend in the *Deinococcus* genomes of genes encoding phosphatases, nucleases, and proteases are predicted to support the formation of Mn^{2+} complexes (Ghosal et al., 2005; Makarova et al., 2001, 2007). This hypothesis is strongly favoured with respect to the growing genetic and functional genomics.

1.6 Anti-oxidant protection in *D. radiodurans* R1

The oxidative damage to the cell is limited by ROS scavenging activity of the cell. The *D. radiodurans* antioxidant defense machinery is active against all three primary reactive oxygen species: hydroxyl radicals (OH•), superoxide radicals (O_2•), and hydrogen peroxide (H_2O_2). The following section briefly describes the repertoire of enzymatic and non enzymatic anti oxidant activity of *D. radiodurans* R1.

1.6.1 Enzymatic protection

D. radiodurans encodes three catalases, four superoxide dismutases (SOD) (Mn-dependent and Cu/Zn-dependent), a cytochrome *c* peroxidase and an iron-dependent peroxidase (Makarova, 2001). *D. radiodurans* is much more resitant to H_2O_2 than is *E. coli*, with a large shoulder in the survival curve (Wang and Schellhorn, 1995). According to data reported by Wang and Schellhorn (1995), the catalase activities during exponential and stationary phases are 127 and 32 times higher those in *E. coli*, respectively. Catalase activity is affected by H_2O_2 (Wang and Schellorn, 1995), ionizing radiation (Tanaka et al., 1996), the addition of manganese (Chou and Tan, 1990), and the growth phase (Wang and Schellorn, 1995), with a higher level of catalase activity in stationary phase cells than in exponential phase cells (Wang and Schellorn, 1995). Catalase activity is negatively controlled by the transcriptional regulator DrRRA (Wang et al., 2008) and positively controlled by OxyR (94). DR1998 is induced in response to ionizing radiation (Tanaka et al., 1996; Tanaka et al., 2004).

Among the SOD proteins, Mn-SOD, is constitutively expressed (Lipton et al., 2002). It efficiently eliminates higher $O_2^{\bullet-}$ concentrations than Mn-SODs in *E. coli* and humans due to the more rapid protonation and release of H_2O_2 (Abreu et al., 2008). *D. radiodurans* catalase and superoxide dismutase mutants are sensitive to H_2O_2 and paraquat, respectively, but not to ionizing radiation at doses lower than 16 kGy (Markillie et al., 1999). The absence of a strong positive correlation between catalase activity and (i) the MIC of H_2O_2 or (ii) ionizing radiation resistance across *Deinococcus* species suggests that other (nonenzymatic) antioxidants (such as manganese complexes) contribute to the scavenging of H_2O_2 (Shashidhar et al., 2010). Fig.1.6 summarises the regulation of catalase and SOD and catalase in DR1.

D. radiodurans also encodes other oxidative defense proteins, such as glutaredoxin, thioredoxin, thioredoxin reductase, and alkyl hydroperoxide reductase, while glutathione, glutathione reductase, and glutathione peroxidase are absent (White et al., 1999). In *E. coli*, the alkyl hydroperoxide reductase is the primary scavenger of endogenous H_2O_2 (Seaver and Imaly, 2001). Thioredoxin reduces oxidized cysteines in proteins and is reverted from its oxidized form by thioredoxin reductase in an NADPH-dependent reaction (Obeiro et al., 2010; Seo and Lee, 2006). *D.radiodurans* also possesses two peptide methionine sulfoxide reductases,

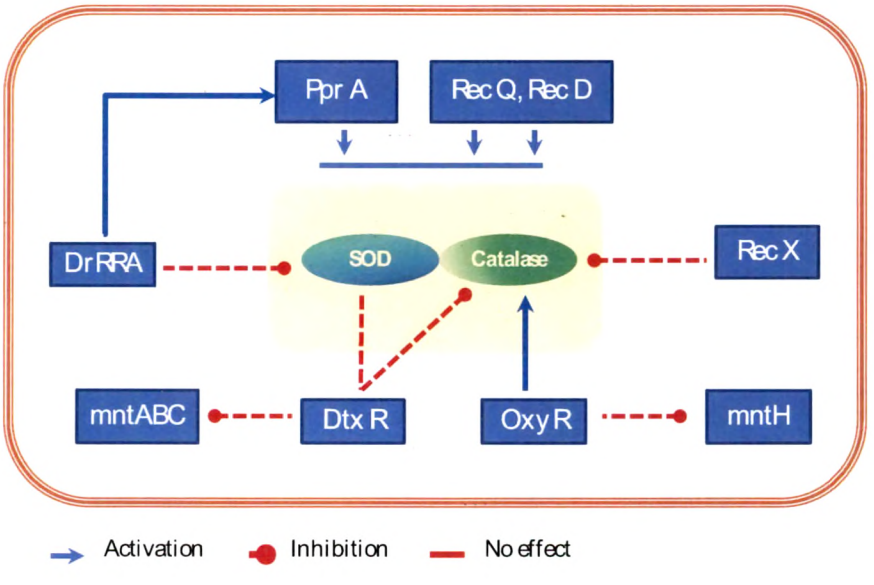

Fig. 1.6 Regulation of ROS combating enzyme in *D. radiodurans* R1. Compiled from Slade and Radman (2011)

MsrA and MsrB (Omelchenko et. al, 2005), which are important for the reduction of oxidized methionine in proteins. MsrA is transcriptionally induced following ionizing radiation (Tanaka et al., 2004).

1.6.2 Pyrroloquinoline–quinone (PQQ)

The role of *pqq* E was postulated as an inducer of a DNA repair and homologous recombination protein kinase, involved in radiation resistance and double strand break repair in DR1 (Rajpurohit et al., 2008). Heterologous expression of deinococcal pyrroloquinoline–quinone (PQQ), a redox factor for several dehydrogenases, in *E.coli* enhanced the catalase and SOD activity in the host *E.coli* (Khairnar et al., 2003). PQQ neutralizes the ROS by directly reacting with them through single electron transfer mechanism and theadducts, thus formed, are non-oxidant in nature. PQQ also functions in a concentration dependent manner in protecting the proteins and DNA from the oxidative damage caused by γ radiation in solution, suggesting a role of PQQ as a radioprotector. *pqq*E mutants of the DR1 and sensitive to IR as well as mitomycin C induced damage to DNA and exhibit a retarded recovery from radiation as opposed to the wild type cells (Misra et al., 2004).

1.6.3 Dps (DNA protection during starvation protein)

The effect of Dps on survival of the cells recovering from high dosages of H_2O_2 suggests that DNA damage might be one of the sites of toxic lesions caused by high concentrations of H_2O_2 as it is believed to be caused by Fe^{2+} tightly bound to the DNA bases and phosphodiester backbone (Martinez and Kolter, 1997). Thus, prevention of coordination of Fe^{2+} atoms with the DNA by Dps binding could explain protection from both direct and indirect (through Fenton's chemistry) mode of killing. Alternatively Dps could act by scavenging hydroxyl radicals in the vicinity of DNA. Such a mechanism has been proposed to explain the decreased sensitivity to oxidative DNA damage of chromatin. *D. radiodurans* encodes two Dps homologs, Dps1 (DR2263) and Dps2 (DRB0092). A dimeric form of Dps1 protects DNA from hydroxyl radical cleavage (Groove and Wilkinson, 2008), which may also be true for Dps2, as the *dps2* mutant is sensitive to H_2O_2 (Slade and Radman, 2011). Both Dps1 and Dps2 are induced in response to ionizing radiation (Liu et al., 2003; Tanaka et al., 2004).

1.6.4 Cartenoids

Most of the deinococci are pigmented pigmentation ranging from red to pink to orangish-red. Deinoxanthine is the prominent cartenoid present in *D. radiodurans*. Deinoxanthin act as more efficient scavenger of H_2O_2 and singlet oxygen than lycopene, β-carotene and lutein because of their extended conjugated double bonds (Tian et al., 2007). DcrtB, mutant of *D. radiodurans* wherin carotenoid biosynthesis was blocked, showed enhanced protein oxidation following treatment with H_2O_2 indicating that the intracellular proteins in the cell without carotenoids were more susceptible to oxidative damage compared to the wild-type cell (Tian et al., 2009).

1.6.5 Manganese complex and its effect on radiation induced oxidative stress

The ability of organic complexes of Mn^{2+} was demonstrated first by Berlett et al., (1990). The first report of Mn^{2+} accumulation in *D. radiodurans* was by Leibowitz et al. (1976), who demonstrated that *D. radiodurans* contained approximately 100 times more Mn than *E. coli* when grown in a defined minimal medium (DMM). Later Daly et al., (2004) established that all radiation resistant bacteria accumulated higher concentration of Mn^{2+} as opposed to Fe^{2+} and therefore reflected in higher Mn/Fe ratio. The same observation was also extended for desiccation resistant bacteria (Daly et al., 2004).

Compared to most organisms, proteins in *D. radiodurans* are highly protected from ROS, but lose their resistance when purified from the cells (Daly et al., 2007). In contrast, DNA in *D. radiodurans* R1 is damaged with essentially the same dose dependence as in all prokaryotic and eukaryotic cells examined (Daly et al., 2004; Daly, 2009; Gladyshev and Meselson, 2008). When orthophosphate (13 mM), Mn^{2+} (200 mM), and peptides (3 mM) were combined in vitro at concentrations approximating those in *D. radiodurans*, the mixture preserved the activity of *Bam* HI and glutamine synthetase exposed to 17.5 kGy, but did not significantly protect DNA. 17.5 kGy represents the outer limits of *D. radiodurans* survival and breaks its 4–8 haploid genomes per cell into 1,000–2,000 DSB fragments (Daly et al., 2011). Thus, protein protection mediated by small Mn^{2+} complexes provides an explanation for the large shoulders in ionizing radiation dose-response curves of *D. radiodurans* survival which distinguishes them from radiosensitive organisms (Daly et al., 2004).

Based on whole-genome comparisons, there is a remarkable abundance in DR1 of genes encoding catabolic enzymes including phosphatases, nucleases and proteases, which would be expected to give rise to the sorts of small molecules accumulated in

the DR1 ultrafiltrate.(Krisko and Radman, 2010; Daly et al., 2010) *D. radiodurans* exposed to ionizing radiation produces an intracellular pool of nucleotides which are subsequently converted to nucleosides (Battista, 1997) that form complex with Mn^{2+} that prevent PC during irradiation induced ROS.

Neutron activation analysis (NAA) reveals that, *D. radiodurans* R1 accumulated a total of aprox. $0.29610E^{-18}$ mol Mn/cell (aprox. 1.86105 Mn atoms/cell; or ,4 mM Mn, given a cell volume of 6.561022 mm^3). When *D. radiodurans* R1 was incubated in minimal medium containing the radioisotope ^{54}Mn, the cells accumulated approximately 3 mM Mn (Daly et al., 2004). X-ray fluorescence (XRF) microspectroscopy revealed that Mn^{2+} is distributed throughout DR1 cells grown in TGY, but with regional intracellular Mn^{2+} concentrations ranging from 0.4 to 3 mM (Daly et al., 2007). *D. radiodurans* lacks most of the Fe-chelating and Fe-transport systems identified in IR-sensitive bacteria (Ghosal et al., 2005; Makarova et al., 2007); most iron in *D. radiodurans* is sequestered outside of the cytosol in the septum between dividing cells (Fig. 1.7) (Daly et al., 2007).

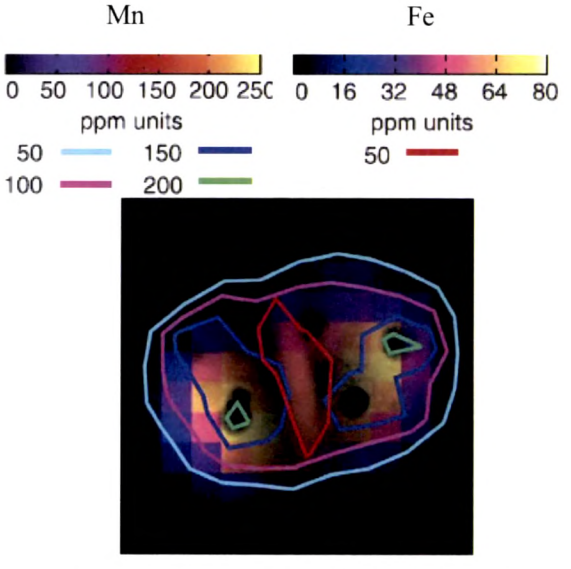

Fig. 1.7 XRF image analysis demonstrating the distribution of Mn^{2+} and Fe^{2+} in D. radiodurans R1 (Daly et al., 2007)

Intracellular accumulation of Mn^{2+} and its complexation with small molecules such as the peptides, orthophosphate has been reported in *D. radiodurans* and is forwarded as the main mechanism of radiation resistant in *Deinococcus*. Not only in *Deinococcus* but also in other bacteria Mn^{2+} play an important role in survival such

as those in *Bacillus* spore formation, pathogenesis of streptococci, and survival of facultative aerobes such as *Lactobacillus plantarum* (Archibald and Fridrovich, 1981). Manganese is the only metal involved in the water oxidizing complex of photosynthetic organisms (Kehres and Maguire, 2003), and involved in the enzymatic dismutation of superoxide radical anion. The discovery of Mn^{2+} as a substitute of SOD to scavenge O_2^- in Lactobacillus and *Neiserria* has diverted the attention to the importance of Mn^{2+} in bacterial systems (Jakubovics and Jenkinson, 2000). Some well known protein that are Mn^{2+} dependent are summarised in Table 1.4.

Table 1.4: Mn $^{2+}$ dependent pathways in bacteria (Jakubovics and Jenkinson, 2001)

Process /pathway	Enzyme /protein	Reference
	Mn^{2+} dependent deinococcal proteins	
DNA repair	UVDE endonuclese	Evans and Mosley, 1985
	DNA polymerase X	Blasius et al., 2006
	NAD dependent DNA ligase	Blasius et al., 2006
RNA repair	RNA ligase	Martins and Shumann, 2004
Nucleic acid metabolism	Nudix hydrolases	Fisher et al.,2006
Oxidative stress response	Mn-SOD	Juan et al., 1991
Sugar metabolism	Fructose-1,6 biphosphate aldolase	Zhang et al., 2006
	Mn^{2+} dependent proteins in bacteria	
Photosynthesis	Mn stabilising protein(PSII-O)	Morgan et al., (1998)
Gluconeogenesis	PEP synthase Pyruvate Carboxylase	Chao et al., (1993) Mukhopadhay et al., (1998)
Glycolysis	3-Phosphoglyerate mutase	Chandler et al., (1998)
Sugar metabolism	6-Phospho-β-glucosidase L-Fucose isomerase	Thompson et al., (1999) Seamann & Schulz, (1997)

Amino acid metabolism	Arginase Glutamine synthetase Threonine 3-dehydrogenase	Sekowaka et al., (2000) Abell et al., (1995) Chen et al., (1995)
Peptide cleavage	Aminopeptidase P	Yocum & Pecoraro, 1999
Nucleic acid degradation	Ribo nuclease H III Endonuclease IV	Ohtani et al., 2000 Hosfeld et al., 1999
Siganl transduction	Serine /threonine protein and phosphatases 1 and 2	Missiakas & Raina, 1997
Stringent response	(p)ppGpp3 Pyrophosphohydrolase	Rao et al., 1998
Oxidative stress response	Magni- catalase Mn-SOD	Whittaker et al., 1999 Fridovich, 1995

1.7 *Deinococcus* as candidate for bioremediation

Nuclear waste sites were generated during the cold war and continue to grow in number due to the use of nuclear power to generate electricity. In the United States alone , buried radioactive wastes is estimated to be cover an area of (3 X 10^6 m^3) that has contaminated about 7 X 10^7 m^3 of surface and subsurface soils and about 3 X 10^{12} dm^3 of groundwater. The most common contaminants from DOE wastes that have been found in ground and ground waters include the radionuclides ^{235}uranium (γ, α) E , ^{238}plutonium (α) E, ^{99}technetium (β^-) E, ^{90}strontium (β^-) E, and ^{137}cesium (γ, β^-)E, and the metals chromium, lead and mercury along with a myriad of toxic organic compounds (e.g. toluene and trichloroethylene (TCE) (Daly, 2000).

These vast waste sites are therefore potential targets for less expensive *in situ* bioremediation technologies utilizing specialized microorganisms that can detoxify both metallic and organic contaminants. However, the utility of microbiological methods for the primary treatment of highly radioactive environmental wastes will largely be determined by the ability of microorganisms catalyzing the desired function(s) to survive and function under radiation stress. and non-pathogenicity of the culture.

Several bacteria such a s *Shwenella* sp., *Pseudomonas* spp. are well known for the capacity to reduce variety of metals and mineralize several organic compounds respectively but are radiation sensitive. Therefore for the cleanup of the nuclear waste sites the radiation resistant microorganisms become the obvious choice. Most radiation-resistant bacteria that have been reported are spore-formers and are not remarkably radiation resistant when growing vegetatively; many of them are pathogens such as *Enterococcus faecium* and *Alcaligenes* spp., and most of them lack a developed system for genetic manipulation (Daly, 2000). Bacteria belonging to the family *Deinococcaceae* are not only the most radiation-resistant organisms discovered, but they are vegetative, easily cultured, and nonpathogenic. Other radiation resistant bacteria that have been reported are not studied with respect to their potential as bioremediation.Although several radition resistant bacterial isolates have been described, the ease of genetic manipulation of the members of genus *Deinococcus* further affirms the use of the deinococci for bioremediation of the radioactive waste sites.

1.8 Engineering *Deinococcus radiodurans* for bioremediation

Most of the deinococci can grow in presence of 6000 rad/h comparable to those found at several nuclear waste sites. The ability to grow in presence of chronic radiation and ease of transformability of *D. radiodurans* allows engineering for bioremediation at nuclear waste sites. The engineered strain of *D. radiodurans* R1 expressing Mer A not only tolerates 30-50 μM Hg^{2+} but also reduces Hg^{2+} to elemental Hg° (Brim et al., 2000). Similarly, *D.geothermalis*, a thermophile, has been engineered with *mer*A and finds applicability at nuclear waste where the higher temperatures prevail (Brim et al., 2003). Quin et al., (2005) transformed *D. radiodurans* R1 with the metal binding domain of Mer R, regulatory protein,to effectively increase the tolerance of the transformed strain to Hg^{2+}. Appukuttan et al., (2006) successfully transformed *D. radiodurans* R1 with *pho* N from local isolate of *S.enteritica* serovar *typhimurium* for precipitation of Uranium to Uranium phosphate from dilute nuclear waste.

Apart from transforming *D. radiodurans* for metal remediation, it has been engineered with the toluene dioxygenase genes (*todC1C2BA*) of *P. putida*. During chronic irradiation, these strains were able to oxidize toluene, chlorobenzene, and 3, 4-dichloro-1-butene (Lange et al., 1998).

Scope of the thesis

The genus *Deinooccus* is rapidly expanding with a large majority of new species isolated by application of γ rays as a selective pressure. Deinococci have been the major focus of study with respect to the mechanism of its ionising radiation resistance. Also deinococci have been forwarded as the major candidate for bioremediation at the nuclear waste sites by its virtue to withstand with very high doses of ionising radiation. However such sites are often contaminated with other pollutants of particular importance are metal contaminants, which could have profound effect on deinococci.

The present study encompasses,

* The development of a molecular method based on 16S rRNA gene for the detection of deinocci from environment without using ionising radiation as selection pressure.

* The investigation of h eavy metal tolerance of the radition resistant bacteria and the mechanism of Cd^{2+} toxicity in *Deinococcus radiodurans* R1.

* Cloning and expression of synthetic metallotheionin and a prokaryotic metallotheionin, *smt* A in *Deinococcus radiodurans* R1.