General discussion
GENERAL DISCUSSION

*D. radiodurans* can survive the extremely high dose of 5000 Gy without loss of viability (Moseley and Mattingly, 1971). The radioresistance of *D. radiodurans* cannot be related to prevention of DNA damage, because DNA double-strand breaks are formed at the same rate in radiation sensitive bacteria and *D. radiodurans* when cells are irradiated under identical conditions (Gerard et al., 2001). A 5000 Gy dose introduces approximately 200 DNA double-strand breaks per *D. radiodurans* genome equivalent (Cox and Battista, 2005). In comparison only a few DNA double-strand breaks can kill an *E. coli* cell (Krasin and Hutchinson, 1977). Irradiated *D. radiodurans* cultures show a dose-dependent growth lag. The isolation of Slow recovery mutants (Mattimore et al., 1995) presenting no defect in DSB repair but a delay in restarting cell growth after irradiation suggests that *D. radiodurans* possesses mechanisms for sensing the completion of DNA repair to allow reinitiation of replication and/or of cell division (Battista, 1997; Battista et al., 1999). All the available evidence indicates that efficient repair of DNA damage is responsible for the radioresistance. DNA repair in this bacterium takes place in two phases, RecA independent and RecA dependent phases. The involvement of recA in DNA repair is observed in other bacteria, but the recA independent phase is unique to *D. radiodurans* (Daly and Minton, 1996). This pathway is active during and immediately after DNA damage and before the onset of recA-dependent repair. During this recA-independent phase, about one third of the 150-200 DSBs per chromosome could be repaired following exposure of 1.75 megarads (Zahradka et al., 2006).

During RecA independent repair phase several important proteins are known to get induced, which includes detoxification proteins, chaperons, transcription/translation processing factors etc. In *D. radiodurans*, 3 predicted superoxide dismutases (DR1279, DR1546, and DRA0202) and 3 predicted catalases (DR1998, DRA0259, and DRA0146) (Omelchenko et al., 2005), which can protect biomolecules from ROS-mediated damage, are induced.
after exposure to ionizing radiation. The multiple chaperones that are induced after irradiation (Tanaka et al., 2004) restore activity of conformationally damaged proteins. Ohba et al., (2005) observed that the pprA protein increased in the cells exposed to 2 kGy gamma radiation followed by 30 min post irradiation recovery. It is previously known that the gene product of pprA, bound preferentially to ds DNA with strand breaks, inhibits E. coli exonuclease III activity and stimulates the DNA end joining reaction catalyzed by ATP dependent ligase, suggesting that pprA has radiation induced non homologous end joining property (Narumi et al., 2004).

It has been observed that after exposure to radiation, most of the methylated DNA is present in the degraded fraction (Prasad et al., 2005 Ph.D Thesis) and there is no new DNA synthesis during this phase. During this lag phase in the post irradiation recovery the bacteria assemble, rearrange and put together the DNA fragments in such a way that the genome could be reconstituted without a single mutation. Since methylation is a very specific marker, it could be an important signal that helps in identification/differentiating regions in the DNA, consequently playing play an important role in the process of reassembly of shattered genome.

DNA methylation could be important to D. radiodurans, primarily because it could modulate molecular events due to presence of methylation on specific DNA residues at a given time and secondly, since it might influence activity of certain genes regulating a cascade of events.

The methylation status of DNA by itself might allow certain regulatory protein(s) to bind the DNA and help in DNA repair (Meselson, 1988, Gonzalez-Nicieza et al., 2001) and/reassembly. Certain DNA binding proteins in the recA independent phase are involved in recognition of methylation of fragmented DNA (Desai et al., 2009 Ph.D Thesis) and hence methylation could act as a marker for protein binding for protection of the DNA ends. Further analysis of the specific methylated DNA binding proteins during initial phase of PIR in D. radiodurans could help in better understanding of role of DNA methylation in this organism. Alternatively, since the GC rich genome of D. radiodurans is
extensively repeat sequences, this low complexity DNA would harbour less number coding sequences. In *D. radiodurans*, cytosine methylation could distinguish functionally different regions of the genome. After DNA damage, the more useful regions could be then preferentially repaired by the organism thus increasing the efficiency of repair. DNA methylation is a global regulator and has been shown to control expression of several genes in prokaryotes (Collier *et al.*, 2007, Campellone *et al.*, 2007, Casadesus 2005, Heithoff *et al.*, 1999; Low *et al.*, 2001). It appears that several regulatory proteins (such as Lrp, GutR, H-NS, and OxyR) binding to their regulatory sites depends on the Dam methylation pattern of those sites (Low *et al.*, 2001).

It was observed that important proteins in the recA independent DNA repair phase were less abundant in both DNA adenine- and cytosine -methyltransferase mutants, indicating that in wild type, DNA methylation positively modulates expression of critical genes in *D. radiodurans*. Some of these genes show presence of the conserved sequence that can be methylated at adenine or cytosine residues in it. Presence of methylation sequence in the upstream region of the genes indicates that DNA methylation is involved in the regulation of these genes. This observation strongly supports the hypothesis that expression of important proteins could be regulated by DNA methylation in *D. radiodurans*.

In adenine methyltransferase mutants, proteome was found to be more adversely affected than cytosine methyltransferase mutant, consequently resulting in more severe growth defects associated with adenine methyltransferase mutants. The concomitant loss of functions important in chaperoning, metabolic and stress responsive proteins in both mutants further signifies role of DNA methylation in *D. radiodurans*. Double (knockout) mutant of adenine and cytosine methyltransferase did not survive after first sub-culturing and could not be isolated, further substantiates involvement of DNA methylation in crucial biological events in *D. radiodurans*.

In site of complete replacement of the *DR_0643* ORF in *D. radiodurans* genome, mutant phenotype was only partial, indicating presence of at least
one more functional DNA adenine methyltransferase activity and its corresponding gene in *D. radiodurans*. An analysis of the annotated *D. radiodurans* genome sequence ([http://www.tigr.org/](http://www.tigr.org/)) revealed that this organism possesses *DR_2267*, a DNA modification methyltransferase-related protein gene, which has homology to some of the domains present in N6 methylase. In the *DR::kan* mutants, the residual adenine methyltransferase activity and genomic DNA 6mA content may be contribution of methylase activity of *DR_2267* or related protein. It is, however, necessary to confirm whether the *DR_2267* ORF codes for an active methyltransferase enzyme.

DNA methylation in *D. radiodurans* by itself is not enough to explain the peculiar DNA reorganisation and repair characteristic of this organism, neither does it explain the unusual biology of *D. radiodurans*. DNA methylation is also present in other bacteria irrespective of their radiosensitivity or non extremophilic phenotypes. However, the events controlled by DNA methylation, which contribute to unique DNA repair mechanism and radiation resistance property of *Deinococcus radiodurans*, need further in depth analysis in light of the observations reported in this work.