2007

The Effects of recQ, uvrD, and recD Mutants of Deinococcus radiodurans on Resistance to DNA Damaging Agents

Stephannie Gauthier Ruiz
Louisiana State University and Agricultural and Mechanical College

Follow this and additional works at: https://digitalcommons.lsu.edu/gradschool_theses

Recommended Citation
https://digitalcommons.lsu.edu/gradschool_theses/3734
THE EFFECTS OF recQ, uvrD, AND recD MUTANTS OF DEINOCOCCUS RADIODURANS ON RESISTANCE TO DNA DAMAGING AGENTS

A Thesis
Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College In partial fulfillment of the requirements for the degree of Master of Science

In

The Department of Biological Sciences

By
Stephannie Gauthier Ruiz
B.S., University of Louisiana at Lafayette, 2004
May, 2008
# TABLE OF CONTENTS

ABSTRACT ........................................................................................................... iii

CHAPTER 1 INTRODUCTION....................................................................................1
  1.1 Background ....................................................................................................1
  1.2 DNA Damage ...............................................................................................4
  1.3 What Is Known of Deinococcal Repair? ......................................................5
  1.4 Role of Helicases in Repair for E. coli .......................................................8
  1.5 Rationale for Conducting Studies ............................................................11
  1.6 Methods ....................................................................................................12

CHAPTER 2 RESULTS ..........................................................................................25
  2.1 The Construction and Characterization of the ΔrecD Strain SG4 .............25
  2.2 The Construction and Characterization of the ΔrecQ Strain SG5 ............30
  2.3 The Construction and Characterization of the ΔrecQ, ΔuvrD Strain SG8-2...37
  2.4 Transformation Efficiencies Using ΔrecD Strain SG4 ...............................42
  2.5 Results Summary .......................................................................................44
  2.6 Discussion ..................................................................................................45

REFERENCES ....................................................................................................50

VITA ...................................................................................................................59
ABSTRACT

The genes encoding the putative nuclease RecD and the helicase RecQ were deleted from *Deinococcus radiodurans* R1 and replaced with constructs conferring hygromycin or spectinomycin resistance. The ∆recD and ∆recQ strains were found to be similar to wild type R1 strain in resistance to gamma irradiation. In contrast to the single mutant strains, the ∆uvrD, ∆recQ double mutant was found to be extremely radiation sensitive, indicating that these proteins share a complementary activity needed for radioresistance. ∆recD mutants have higher transformation efficiencies than the wild type R1 strains, suggesting an association of RecD with nucleases that degrade single stranded DNA. Mitomycin C and ultraviolet light, DNA damaging agents that generate different patterns of DNA damage relative to ionizing radiation, were also used to induce damage in the wild type strain mutant strains. The ∆uvrD, ∆recQ double mutant exhibited the highest sensitivity to these mutagens, reiterating that RecQ and UvrD have overlapping activities. Hydrogen peroxide increased the sensitivity of the RecQ mutant approximately ten fold, suggesting that RecFOR protein complex may be important in repairing damage mediated by oxygen radicals.
CHAPTER 1 INTRODUCTION

1.1 Background

- **Ionizing Radiation Resistance**

  Ionizing radiation is strong enough remove electrons from atoms to form ions. Gamma radiation reacts with material to form high kinetic energy electrons by three methods: photoelectric, Compton-scatter, and pair-production processes (VanZile, 2004; McCann, 2006). The absorbed dose is the energy exchange from the radiation field to a substance (measured in Gray), which is calculated as energy deposited per unit mass. One Gray (Gy) is 10,000 ergs per gram (VanZile, 2004; McCann, 2006). The resulting ions cause damage to cellular macromolecules by either directly modifying the macromolecule or by generating reactive intermediates (such as the hydroxyl radicals formed in aqueous environments) that in turn modify the macromolecule. Ionizing radiation causes many types of cellular damage, but the most detrimental is the DNA double strand break. This type of damage effectively destroys the genome by causing irreparable damage that ultimately leads to the loss of essential genes.

- **Deinococcus radiodurans**

  Most ionizing radiation resistant organisms are found in domains *Bacteria* and *Archaea* (Rainey *et al*., 2005). The genus *Deinococcus* includes twenty three validly named species (Cox and Battista, 2005). In 1956 a reddish pigmented bacterium was discovered spoiling canned ground horse meat following a sterilizing dose of gamma rays; it was termed *Micrococcus radiodurans* due a phenotypic appearance similar to *Micrococcus roseus* (Anderson *et al*., 1956). The bacterium was designated strain R1, and grew in tetrads as spheres from 1.5 to 3 microns in diameter in aerated TGY broth. Non sporulating pink round colonies were observed on TGY agar and high catalase activity was present (Anderson *et al*., 1956). In 1980, fifty individual strains of *Micrococcus radiodurans* were further characterized along with other radiation...
resistant strains including \textit{Micrococcus radioproteolyticus}, \textit{Micrococcus radiophilus}, and \textit{Micrococcus radiopugnans}, to determine the differences from other \textit{micrococc}al species such as \textit{M. roseus}, \textit{M. agilis}, and \textit{M. luteus} (Brooks et al., 1980). Based on all differences found in 16s rRNA, DNA, fatty acid composition, and cell wall structures between the radiation resistant \textit{micrococc}i and the regular \textit{micrococc}i, a separate genus name was proposed. The family name \textit{Deinococcaceae} is given for the radiation resistant species, and the designation \textit{Deinococcus radiodurans} is given meaning strange or unusual berry that withstands radiation (Brooks and Murray, 1981).

\textit{D. radiodurans} is a nearly ubiquitous bacterium, mainly found in soil (Battista, 1997; Rainey et al., 2005). The deinococcal species can endure dosing as high as 25kGy and recover (Rainey et al., 2005). These amazing bacteria are able to grow normally with a dose of 60Gy per hour (Lipton et al., 2002). \textit{D. radiodurans} is 50-100 times more radiation resistant than \textit{Escherichia coli}, and survives 5,000 to 15,000Gy exposures to ionizing radiation (Smith et al., 1988; Daly et al., 1994; Lipton et al., 2002). The dose needed to kill 63% of an exponentially growing population is approximately 6,000Gy. These high doses of ionizing radiation cause large amounts of double strand breaks in the genomic DNA which these bacteria are able to efficiently repair.

\textit{Deinococcus radiodurans} is non-motile, aerobic, non spore-forming, and exist as tetrads or dyads in broth. The size of the bacterium varies from 1.5 to 3.5µm across the sphere and stains gram positive, however contains a cell envelope concordant with a gram negative bacterium (Battista, 1997). These cells grow best in tryptone-glucose-yeast extract (TGY) media at 30°C. During exponential phase, cells contain 8-10 copies of its genome and 4 copies during stationary phase (Hansen, 1978; Masters, 1991). When cells are grown in TGY, they have the capacity to double in an hour and twenty minutes (Hansen, 1978; Battista, 1997). The genome of
*Deinococcus radiodurans* consists of a 2.68Mb chromosome, a 0.41Mb chromosome, a 0.18Mb megaplasmid, and a 0.045Mb plasmid. Overall, the genome is 3.28Mb and has a high GC content of 66.6% (Lin et al., 1999). The nucleoid is a tightly packaged structure that appears as a single ring within each cell (Zimmerman and Battista, 2006). *Deinococcus radiodurans* is a transformable species and competent throughout exponential phase, similar to *Neisseria meningitidis* (Tigari and Moseley, 1980; White et al., 1999). In 1968, Moseley and Setlow determined that *Deinococcus (Micrococcus) radiodurans* is able to pick up genomic DNA extracted from mutant deinococcal strains resistant to streptomycin to yield resistant transformants (Moseley and Setlow, 1968).

*Deinococcus radiodurans* is highly resistant to desiccation, and a 10% survival rate has been reported after 6 years of desiccation (Battista, 1997). Desiccation introduces numerous double strand breaks to the genome, similar to ionizing radiation induced damage (Mattimore and Battista, 1996; Cox and Battista, 2005). *Deinococcus* does not prevent damage or protect its DNA since the genome of a cell in exponential phase is broken down to 10,000-50,000 base pair fragments (Cox and Battista, 2005; Mattimore and Battista, 1996). In order to provide evidence suggesting that the ability to survive extreme drying is connected to the ability to survive ionizing radiation, a study of 41 ionizing radiation sensitive strains was conducted (Mattimore and Battista, 1996). Each ionizing radiation sensitive strain was also sensitive to desiccation, suggesting that *D. radiodurans* ionizing radiation resistance evolved incidentally as part of an adaptation to repair extensive amounts of double strand breaks from severe drying (Mattimore and Battista, 1996).

Most DNA repair genes encoded by *D. radiodurans* have orthologs found in *E. coli* (Makarova et al., 2001; White et al., 1999; Liu et al., 2003). The number of repair enzymes found so far is fewer than those identified in *E. coli* (Lipton et al., 2002). There are orthologs of
11 genes from the 26 genes involved in *E. coli*’s SOS response, and of those only 7 genes are induced after exposure to radiation in *D. radiodurans* (Makarova *et al.*, 2001; Liu *et al.*, 2003).

### 1.2 DNA Damage

Ionizing radiation causes the ionization of molecules by the deposition of energy into matter. Gamma rays are photons and have three separate reactions that form ions, but the Compton Effect is the most common and happens as the kinetic energy released following the interaction of of x-rays or gamma rays with a substrate expels electrons creating ions. Cells are damaged via the free radicals released once the gamma particle hits a substrate (Cox and Battista, 2005). Ionizing radiation damage is greatly increased by aqueous environments able to produce oxygen free radicals to attack cell membranes, proteins, and DNA (Markillie *et al.*, 1999). Ionizing radiation usually results in modification of the bases in DNA; a less common effect is damage to sugar-phosphate backbone causing a single strand break. Double strand breaks are one of the most detrimental forms of damage to DNA in most bacteria.

Hydrogen peroxide constitutes a reactive oxygen molecule often involved in DNA damage. Hydrogen peroxide produces radicals that cause significant damage to proteins, lipids, carbohydrates and DNA (Friedberg *et al.*, 1996). Hydrogen peroxide alone is not as damaging as the products it forms. These include hydroxyl radical, hydroxide ion, or superoxide, formed once hydrogen peroxide contacts any transition metal like Fe normally present within cells (Henle and Linn, 1997). During the Fenton reaction an iron atom is oxidized as hydrogen peroxide molecule is reduced, forming the unstable hydroxyl radical (Imlay *et al.*, 1988).

Mitomycin C halts DNA synthesis in bacterial cells and prevents cell division (Tomasz *et al.*, 1988; Weissbach and Lisio, 1964). The drug/mutagen is a bioreductive alkylating agent (Weissbach and Lisio, 1964; Pan *et al.*, 1986) and acts by ‘cross-linking’ to DNA. Interstrand cross-links in DNA inhibit separation of the DNA duplex during semiconservative replication.
Interstrand crosslinks are formed when this bifunctional agent reacts with guanines on opposite strands (Sharma et al., 1994).

1.3 What Is Known of Deinococcal Repair?

Although there is no evidence of passive protection of DNA following gamma irradiation, Deinococcus appears to have characteristics that passively aid in radioresistance. The organism is multigenomic; Deinococcus carries at least four copies of its genome. This reserve of genetic information can serve as backup material for use in recombinational repair (Cox and Battista, 2005). Deinococcus also sequesters high concentrations of manganese within the cell, which has been shown assist in the recovery from damage induced by irradiation, by reacting with superoxide ions to prevent further damage (Daly et al., 2004).

Nucleotide excision repair functions to remove cross-linked DNA and UV induced lesions by removing adducted DNA, creating a single-stranded gap allowing de novo DNA synthesis to fill in the gap.


Synthesis dependent strand annealing is another pathway of repair for D. radiodurans requiring a double strand break nearby an undamaged identical strand (Cox and Battista, 2005; Lovett, 2006). First, 3\’ overhangs are made on the double strand breaks which invade the homologous region on the undamaged duplex creating a D loop and serve as primers for new DNA synthesis (Fig 1.). Once the ends are sufficiently extended, the newly synthesized strands dissociate and anneal to complementary regions where further synthesis continues until the gaps
are filled and the double strand break is repaired (Cox and Battista, 2005; Haber, 2000; Lovett, 2006).

Zahradka and colleagues detected a form of synthesis dependent strand annealing that they referred to as extended synthesis dependent strand annealing (ESDSA). ESDSA requires large amounts of new DNA synthesis and necessitates two identical strands with double strand breaks at different loci. When dissociated overhanging strands serve as primers and anneal for synthesis of complementary single strands (Zahradka et al., 2006; Jones, 2006).

The mismatch repair system is necessary to avoid mutations incurred during replication and recombination. However mismatch repair is not necessary for repair after DNA damage. Unlike \textit{E. coli} (and many other bacterial genera including \textit{Salmonella}, \textit{Klebsiella} and \textit{Haemophilus}, \textit{Yersinia}, and \textit{Vibrio}) there is neither dam methylase, nor MutH involved in the repair pathway.

\textbf{Figure 1}. Image is taken from Lovett 2006 and depicts steps involved in synthesis dependent strand annealing. The color red denotes old DNA blue indicates newly synthesized DNA.
Deinococcus radiodurans contains the three main genes necessary to code for proteins used in the mismatch repair system such as mutS, mutL and mutU/uvrD. When UvrD is nonfunctional, cells become sensitive to a variety of mutagens, thus implicating other roles such as UvrABC pathway which mediates nucleotide excision repair necessary for removing crosslinks and mutations acquired from exposure to ultraviolet irradiation (Menecier et al., 2004; Minton, 1994). Disrupting the uvrD gene yields a mutator strain, indicating that mismatch repair must function in Deinococcus radiodurans (Menecier et al., 2004). Mutation frequencies in UvrD mutants were determined to be $8.3 \times 10^{-8}$ as compared to wild type at $1.5 \times 10^{-8}$ (Kim et al., 2004).

Deinococcus radiodurans lacks a recBCD pathway for homologous recombination, which is present in many bacteria where it is necessary for repair of double strand breaks. The proteins RecB and RecC have not been found in D. radiodurans, however RecFOR proteins are present. In E. coli the RecBCD trimer uses helicase and nuclease activity to process double strand breaks and load RecA onto single stranded DNA for homologous recombinational repair (Dillingham et al., 2003). Apparently, the RecFOR pathway is more important in Deinococcus for homologous recombinational repair and mending of halted replication forks. Proteins RecF, RecO, RecR, RecQ 3’-5’ helicase and RecJ 5’-3’ nuclease are involved in this repair pathway (Leiros et al., 2005). Although this protein plays an important role in recovering stalled replication forks, helicase RecQ does not appear to be induced after exposure to DNA damaging agents (Lipton et al., 2002; Liu et al., 2003).

The RecD protein is the only part of the RecBCD complex expressed by Deinococcus radiodurans, and it appears to function independently. The recD gene found in Deinococcus is most similar to its homologues found in Bacillus subtilis and Chlamydia whose RecD proteins also function independently in the absence of a RecBCD complex (White et al., 1999; Makarova
RecD has been expressed and purified and in vitro it unwinds short lengths of double stranded DNA fragments with 5’ single stranded tails. The protein seems to have low processivity because it dissociates after unwinding about 20 base pairs, and may require other proteins in vivo to increase processivity (Wang and Julin, 2004).

1.4 Role of Helicases in Repair for *E. coli*

UvrD or DNA helicase II functions to unwind DNA in a 3’ to 5’ direction, and is implicated to have a role in methyl directed mismatch repair, nucleotide excision repair, and possibly in replication and recombination. After DNA damage, UvrD is highly induced, and when concentrations are high in vitro this protein is able to fully unwind long segments of DNA when blunt ends or a nick are present (Runyon et al., 1990; Runyon and Lohman, 1989). Non-homologous recombination in wild type strains is thought to be prevented by a functioning mismatch repair system where recombination is halted when mismatches are detected in the invading strand (Zhang et al., 2006). Deletion of the *uvrD* gene results in a 250 fold rise in the spontaneous mutation rate in *Escherichia coli* (Zhang et al., 1998).

RecQ is a ubiquitous helicase conserved from bacteria to humans (Hishada et al., 2004). In *Escherichia coli*, RecQ functions as an ATP dependent helicase unwinding from 3’ to 5’, and is thought to function as a monomer (Zhang et al., 2006). This helicase is highly processive; the rate of unwinding is very fast with an unwinding step size of 4 base pairs (Zhang et al., 2006). RecQ may function independently of RecFOR to generate ssDNA at a stalled replication fork in order to stimulate the SOS response (Hishada et al., 2004). Helicase RecQ will unwind large segments of DNA in the presence of single strand binding protein, but does not load RecA onto the unwound strand (which is RecFOR’s purpose) (Kowalczykowski, 2000).

RecFOR pathway for homologous recombination requires RecQ and is involved in the repair of gapped DNA, the preservation of the newly synthesized strand at a halted replication
fork, and the recommencement of halted replication forks (Ishino et al., 2006). The RecFOR pathway is not well understood as its assembly mechanism is not completely elucidated nor is the molecular ratio of the assembly known (Morimatsu and Kowalczykowski, 2003). The RecFOR protein complex mediates the repair of UV induced lesions, ssDNA gaps, plasmid recombination, and recombination via conjugation at locations far from the double stranded ends (Morimatsu and Kowalczykowski, 2003). Recombination is initiated by first processing blunt ends of double strand DNA breaks using helicase RecQ to unwind and RecJ nuclease to digest and create a free 3’ single stranded end (Kowalczykowski, 2000).

Double strand breaks yield one of two outcomes: a paradoxical role of either initiating recombination or causing cell death. Homologous recombination is mediated by only two complexes in Escherichia coli. The RecFOR protein complex specializes in single strand gaps whereby the RecBCD protein complex specializes in double strand breaks; however RecFOR functions to repair double strand breaks when RecBCD is inactivated. When RecBCD is inactivated, RecQ helicase functions to unwind and cause recombination 75% of the time and the other 25% of activity resumes by UvrD helicase and HelD (Kowalczykowski, 2000). Both of these pathways function as a backup system for the other in addition to specializing in separate repair functions.

The RecD protein is a 58KDa polypeptide and is considered to be the essential third subunit of exonuclease V (RecBCD). When RecD is removed from the complex, nuclease activity decreases, and recombination rates increase occurring independently of chi sites known as recombination sites (Amundsen et al., 2000; Kowalczykowski et al., 1994). As a part of the complex, high levels of nuclease activity occurs when incubated with either single or double stranded DNA (Chen et al., 1997). Wild type Escherichia coli strains are not naturally transformable when cells are mixed with linear fragments of DNA. ΔrecD mutants become
highly transformable with linear plasmid DNA, indicative of the robust nuclease activity RecD provides when complexed with RecBC (Russell *et al*., 1989; Courcelle *et al*., 1997). Protein RecD plays an essential role as the main nuclease in *Escherichia coli*, since ∆recBC mutants are inadequate for digesting double stranded DNA substrates, and processing for repair is not as efficient (Kuzminov and Stahl, 1999).

In *Escherichia coli*, the most important role of exonuclease V is to process double strand breaks to form ssDNA to bind RecA, and initiate homologous recombination. This is essential because any unrepaired double strand break is lethal (Morimatsu and Kowalczykowski, 2003; Kowalczykowski, 2000; Ishino *et al*., 2006). The RecBCD protein complex is a heterotrimer composed of subunits of RecB, RecC, and RecD (Kowalczykowski, 2000; Anderson *et al*., 1997; Ivancic-Bace *et al*., 2003). The entire RecBCD complex is 330kDa, and functions to unwind and digest dsDNA, and load RecA onto ssDNA (Ishino *et al*., 2006). The RecBCD repair pathway has a variety of functions in the cell including acting as: ssDNA exonuclease, ssDNA endonuclease, dsDNA exonuclease, DNA dependent ATPase, forming chi specific fragments, and DNA a helicase (Kowalczykowski, 2000; Ivancic-Bace *et al*., 2005). In addition, exonuclease V degrades 90% of foreign DNA that enters the *Escherichia coli* cell (Kowalczykowski, 2000).

RecJ and RecD are known to be 5’ to 3’ exonucleases (Lloyd and Buckmam, 1991). It was recently found that although the ∆recD deficient RecBC complex lacks nuclease activity; the proficiency of recombination and repair remains because of the activity of other 5’ to 3’ nucleases such as RecJ and exonuclease VII, which compensate for the activity of exonuclease RecD. Mutants deficient in all three 5’-3’ nucleases lose recombination proficiency (Dermic *et al*., 2006).
Recent reviews provide strong evidence indicating that protein RecD functions as a 5’ to 3’ helicase in addition to its previously found nuclease role (Dillingham et al., 2003; Taylor and Smith, 2003; Singleton et al., 2004; Ishino et al., 2006). Protein RecD contains super family 1 helicase domains, and exhibits DNA dependent ATPase activity (Dillingham et al., 2003; Taylor and Smith, 2003). The complex roles that each enzyme plays in the RecBCD pathway (such as cleavage), may further be related to other non helicase domains that have yet to be found in the three subunits (Dillingham et al., 2003). The lack of nuclease activity in a RecD subunit alone may indicate the requirement for direct contact with subunits RecB and RecD to stimulate the nuclease function of RecD in order to prevent nonspecific DNA degradation (Taylor and Smith, 2003).

1.5 Rationale for Conducting Studies

RecD, RecQ, and UvrD are three important repair proteins found in *E. coli*, and were chosen as target proteins in *Deinococcus radiodurans* in order to determine their importance in DNA damage repair in this species. These genes were inactivated to gain insight into the roles these proteins play in this species ability to withstand DNA damage. In *Deinococcus radiodurans*, protein RecD is the only homologue of the RecBCD complex found in *Escherichia coli*. The protein RecD in *D. radiodurans* is a helicase and a putative nuclease. A *recD* gene replacement mutant was created in order to elucidate the function of the protein in repair after exposure to different DNA damaging agents and to test for possible nuclease activity by transformation assays. In *D. radiodurans*, RecD protein seems to play a minimal role in repair and decreases transformation efficiency, a similar effect found in *E. coli* ∆*recD* mutants.

UvrD and RecQ are helicases that unwind DNA from 3’ to 5’ direction. UvrD functions in mismatch repair and in UvrABC nucleotide excision repair in *E. coli*. In *Deinococcus*, nucleotide excision repair is mediated by protein complex UvrABC. The RecFOR repair
pathway is important in *Deinococcus* as it is believed to be the main pathway of double strand break repair, requiring RecQ helicase and RecJ nuclease.

In *Deinococcus radiodurans*, it is known that a single ∆uvrD mutant is insensitive to ultraviolet and ionizing radiation (Chris Davis Guillot, unpublished data). In order to determine whether these helicases in *Deinococcus* were able to complement each other in different repair pathways single and double mutants were created to test the effect on survival following DNA damage.

### 1.6 Methods

*Deinococcus* strains were all grown in tryptone-glucose-yeast extract (TGY) broth or agar (0.5% tryptone, 0.3% yeast extract, 0.1% glucose) at 30°C broths with agitation at 200rpm. Solid media plates were grown without agitation. All *E. coli* strains were grown in Luria-Bertani (LB) broth or plates (1% tryptone, 1%NaCl, 0.5% yeast extract) at 37°C with agitation for broth. Frozen stocks were made of all strains and consist of 150µL dimethyl sulfoxide and 850µL of culture.

### Table 1. Strains used in all studies.

<table>
<thead>
<tr>
<th>Strain Name</th>
<th>Bacteria</th>
<th>Phenotype</th>
<th>Drug Cassette</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1 ATCC 13939</td>
<td><em>Deinococcus radiodurans</em></td>
<td>Wild type</td>
<td>None</td>
</tr>
<tr>
<td>SG4</td>
<td><em>Deinococcus radiodurans</em></td>
<td>recD</td>
<td>Hygromycin</td>
</tr>
<tr>
<td>SG5</td>
<td><em>Deinococcus radiodurans</em></td>
<td>recQ</td>
<td>Spectinomycin</td>
</tr>
<tr>
<td>CD571</td>
<td><em>Deinococcus radiodurans</em></td>
<td>uvrD, uvrA</td>
<td>Chloramphenicol</td>
</tr>
<tr>
<td>SG8-2</td>
<td><em>Deinococcus radiodurans</em></td>
<td>uvrD, recQ</td>
<td>Chloramphenicol, Spectinomycin</td>
</tr>
<tr>
<td>pSG11</td>
<td><em>Escherichia coli</em></td>
<td>Upstream HYG downstream recD</td>
<td>Hygromycin</td>
</tr>
<tr>
<td>pSG27</td>
<td><em>Escherichia coli</em></td>
<td>Upstream CAT downstream recJ</td>
<td>Chloramphenicol</td>
</tr>
<tr>
<td></td>
<td>Escherichia coli</td>
<td>Drug cassette only</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>-----------------</td>
<td>--------------------</td>
<td>----------</td>
</tr>
<tr>
<td>pTNK104</td>
<td></td>
<td></td>
<td>Hygromycin</td>
</tr>
<tr>
<td>pTNK101</td>
<td></td>
<td></td>
<td>Chloramphenicol</td>
</tr>
<tr>
<td>Pino103-2</td>
<td></td>
<td>Upstream Spec</td>
<td>Spectinomycin</td>
</tr>
<tr>
<td></td>
<td>downstream</td>
<td>recQ</td>
<td></td>
</tr>
</tbody>
</table>

- **Chromosomal DNA Purification from D. radiodurans**

  Strains are inoculated from frozen stocks into 4mL tubes of TGY broth, and are allowed to grow for 2 days until cells reach stationary phase. Every *Deinococcal* culture is incubated in the Innova™ 4300 incubator shaker at 30°C with agitation at 200 rpms (New Brunswick Scientific). Cultures are separated into 2mL microcentrifuge tubes, and then spun down for 5 minutes at 5x1000 rpms using an Eppendorf Centrifuge model 5415 D. The supernatant is poured off and cells are resuspended by vortexing in 400mL of 70% ethanol to remove the outer membrane. Cells are centrifuged again, and the ethanol is drained off to add 150µL distilled de-ionized water on ice and 300µL of SDS-EB buffer (Sodium Dodecyl Sulfate-Elution Buffer: 2% SDS, 400 mM NaCL, 40 mM EDTA, 100 mM Tris-HCl, pH 8.0). Tubes are vortexed after water and buffer are added and 350µL phenol/Choroform isoamyl alcohol (1:1) is used to extract cellular debris by inverting. The extraction is repeated 2-3 times. Phases are separated by centrifugation for 5 minutes at 5rpms and aqueous solution is added to a new tube after each extraction. One last extraction is performed by using 300µL chloroform isoamyl alcohol (24:1), centrifuging and transferring aqueous phase to new tube. To precipitate the DNA, 2 volumes of absolute ethanol is used, then tubes are inverted to mix and placed on ice for 30 minutes. The tubes are then centrifuged for 10 minutes at 13.2x1000 rpms and the pellet is washed once with 200µL of 70% ethanol. The microcentrifuge tube cap is then opened, inverted onto chemwipe tissues, and allowed to dry for 15 to 20 minutes. Pellet is then resuspended in 40µL Qiagen elution buffer (10mM Tris–HCl).
• **PCR Conditions**

Taq polymerase with standard 10X Taq buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl\(_2\), pH 8.3) and 10mM deoxynucleotide solution mix is used from New England Biolabs. dNTP solutions are diluted to 2.5mM and are stored in separate tubes. All primers are ordered from Integrated DNA technologies, Inc. and are resuspended in deionized water for a 200mM concentration, then are diluted to 10µM concentration. All PCR reactions took place with a max volume of 20µL, with 20ng of DNA used per reaction. The thermocycler model used is Bio-Rad iCycler. Cycles were repeated 30X for most amplifications.

• **Plasmid Purification from E. coli**

Plasmid-carrying *E. coli* strains are inoculated from a frozen stock and incubated overnight in tubes containing in 4mL of LB broth containing 100µg/mL ampicillin. Qiagen kits were used, specifically the QIAprep Spin Miniprep Kit. The Eppendorf Centrifuge model 5415D was used spin buffers through the filter columns.

• **Protocol for Generating ∆recD Mutant**

The hygromycin drug cassette with the deinococcal pKat promoter was amplified from pTNK104. The fragment upstream from gene *recD* is 1KB of sequence upstream, and the downstream fragment is composed of 800 base pairs directly downstream of the *recD* gene. R1 genomic DNA is used for polymerase chain reaction amplification of upstream and downstream regions at a concentration of 1ng of DNA per microliter of PCR reaction mixture. Final products are excised from gels, and purified using the QIAquick Gel Extraction Kit, and then fragments were cloned into plasmids for easy amplification.
Using the splicing by overlap extension (SOE) technique, tails made on the primers of the drug cassette overlap with upstream and downstream regions (Horton et al., 1989; Howard-Flanders and Bardwell, 1981). Using this technique the upstream region was fused to the drug cassette, and the downstream region was separately fused to the drug cassette. The entire upstream-drug cassette-downstream construct was not made by SOEing, but by ligation using T4 DNA ligase (NEB). Upstream-drug cassette (2,119bp) and Downstream-drug cassette (1,919bp) were digested using restriction endonuclease DraIII to create overlapping ends for ligation. After digestion, these products were then cut out of the gel and purified using the QIAquick Gel Extraction Kit to prepare for ligation.

**Table 2.** List of primers used for generating plasmid and for verifying presence of homozygous *recD* gene knockout.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Tm(°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RecDup1</td>
<td>AGACCGTCCGGGGGTCGC</td>
<td>69</td>
</tr>
<tr>
<td>RecDup2-r</td>
<td>GACGCCTTAAGAGTTCT</td>
<td>55</td>
</tr>
<tr>
<td>RecDdown5b</td>
<td>GCCAGGCAGGATAGCGCGGAAGG</td>
<td>72</td>
</tr>
<tr>
<td>RecDdown800-r</td>
<td>GCCTTGCTCACCACCTCGCC</td>
<td>69</td>
</tr>
<tr>
<td>RecD-Hy7</td>
<td>AGAACTCTTAAGCGTCGAGGGCCTAGGGGCTAGGGCCAT</td>
<td>76</td>
</tr>
<tr>
<td>RecD-Hy8</td>
<td>TATCCTGCTGCCCTAGGCGCGCCGGGGGCGGT</td>
<td>79</td>
</tr>
<tr>
<td>HygDownL</td>
<td>GACCTGCACGGAACCAACAT</td>
<td>60</td>
</tr>
<tr>
<td>DownR</td>
<td>TCCCCATTTCCCCACCCAGACG</td>
<td>60</td>
</tr>
<tr>
<td>RecDa</td>
<td>GGAAGACGATTCGACGCTCT</td>
<td>60</td>
</tr>
<tr>
<td>RecDb</td>
<td>GCAGCCGATCGAGGTACT</td>
<td>60</td>
</tr>
</tbody>
</table>

The Promega PGEM®-T Kit protocol was used to ligate the two fragments overnight, clone into a PGEM®-T vector, and perform the subsequent transformation using JM109 high efficiency competent cells. Transformants were plated on LB/Ampicillin/IPTG/X-Gal plates and white colonies were picked. Plasmid pSG11 contains all 3 pieces in the forward orientation, as determined using restriction endonucleases BstX1 and DraIII ordered from New England Biolabs.
The plasmids were linearized using restriction endonuclease PstI (NEB), and transformed into Deinococcus radiodurans R1. Transformants were plated on TGY plates with 37.5µg/mL hygromycin to select for mutants containing the antibiotic marker. Mutants lacking the recD gene were verified through PCR. Primers were designed to amplify a 1,461 base pair region within the drug cassette, and extending outside of the construct into genomic DNA. This primer was designed to verify the construct underwent homologous recombination at the proper site. Another primer was designed to amplify a short 404 base pair region within the recD gene to verify absence of gene in all mutants.

- **Transformation Protocol**

_Deinococcus_ is grown to exponential phase as described. CaCl from a 1M stock is added to a flask containing about 10mL TGY to get a final concentration of 30mM. The flask is then returned to the Innova™ incubator shaker for 80 minutes. Once proper incubation time is achieved, 1mL of culture is added to a large tube on ice, and 1µg of linearized plasmid or genomic DNA is immediately added. Strains are incubated on ice for 30 minutes prior to diluting with 9mL of fresh TGY broth. The tubes are incubated overnight for 18 hours shaking at 30ºC. The next day cells are plated on TGY media selecting for the appropriate antibiotic resistance marker. In order to obtain homozygous knockouts, isolated colonies are picked and inoculated in TGY broth containing the antibiotic marker, and grown for 48 hours to stationary phase. Frozen permanents are made of these stocks. Next, the strains are then inoculated in TGY broth media without antibiotics and grown to stationary phase. These strains are plated onto TGY plates with the appropriate antibiotic and isolated colonies are picked and grown up in broth also containing the correct antibiotic. More frozen stocks are made after the cells reach stationary phase, and the remaining culture is used for DNA purification for PCR verification of
the absence of the targeted gene. The transformation protocol was adapted from papers (Tigari and Moseley, 1980), and (Moseley and Setlow, 1968).

- **Protocol Used for Ionizing Radiation**

  The Shepherd Co\(^{60}\) gamma-emitting irradiator was used for all ionizing irradiation experiments with supervision from radiation safety officers. Initial dose rates started at 0.2Gy/sec at the center of the irradiator around January of 2006. In September of 2006, it was determined that at 8cm away from the source, a dose rate of 18Gy/min (1,080Gy/hr) was absorbed. Pre-irradiation samples were diluted and plated on plain TGY prior to bringing samples to the irradiator. All samples were irradiated in 1.5mL microcentrifuge tubes, containing roughly 1mL of exponentially growing strains of \textit{D. radiodurans}. Overnight cultures were grown and used to inoculate a 125mL flask containing 10mL of fresh TGY broth where cultures grew for 3 to 4 hours. Absorbance was measured at 600nm using a SmartSpec\textsuperscript{TM} 3000 by Bio Rad until an optical density was reached between 0.1 and 0.2. 600\(\mu\)L of each sample was transferred into a clean 10 x 4 x 45nm cuvette by Sarstedt. All samples of culture were irradiated in Styrofoam racks, with strain R1 serving as a control. After the appropriate dose was given, the samples were removed, lightly vortexed and immediately placed into 10mM magnesium sulfate to stop growth. All cultures were serially diluted to obtain the appropriate dilution factor to obtain adequate isolated colonies for plate counts. TGY plates were used containing no antibiotics and cultures were incubated for 2 to 3 days before colony forming units were counted (Zimmerman and Battista, 2006).

- **Protocol for Generating \textit{ArecQ} Mutant**

  Plasmid Pino 103-2 was obtained from Nicole Pino, the plasmid constructs were digested with restriction endonuclease DraIII to check for 3 distinct bands that should appear, confirming the presence of the upstream-spectinomycin drug cassette-downstream construct. The plasmid
was linearized with restriction endonuclease PstI before transformation and transformants were plated on TGY agar with 75µg/mL spectinomycin. PCR was used to verify whether the \textit{recQ} gene was absent in the transformants, however ambiguous results ensued. Bands were present which could be interpreted as nonspecific amplification in the mutants, or possibly the \textit{recQ} gene amplified to a lesser extent. Another PCR analysis was conducted to determine where the drug cassette was inserted. Primers were designed to start upstream from the gene and amplify into the spectinomycin drug cassette to determine if the recombination occurred in the correct place.

\textbf{Table 3.} List of primers used for generating plasmid and for verifying presence of homozygous \textit{recQ} knockouts.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Tm*(°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RecQb</td>
<td>ATGACCGCTGCTCCTGCGCCGCCCT</td>
<td>55</td>
</tr>
<tr>
<td>RecQa</td>
<td>TTATCCATCGAGCACCCTGTT</td>
<td>70</td>
</tr>
<tr>
<td>SpecUp</td>
<td>AGTTCCGCGCCTCATTG</td>
<td>63</td>
</tr>
<tr>
<td>SpecDown</td>
<td>TGCCCAGGGCATAGACTGTACC</td>
<td>61</td>
</tr>
<tr>
<td>RecQ1</td>
<td>CCGCAGTTCTCCTCCTCCTT</td>
<td>59</td>
</tr>
<tr>
<td>RecQ2</td>
<td>AGCACCTTGAATGCGTTTGCAGCG</td>
<td>61</td>
</tr>
</tbody>
</table>

Southern blot analysis was conducted to determine with certainty whether a homozygous knockout was present in strain SG5. Restriction endonuclease KpnI produced a fragment surrounding the \textit{recQ} gene of 5.6Kb, and endonuclease Clal produced a 4.1Kb fragment surrounding the \textit{recQ} gene. The genomic DNA digest was incubated overnight at 37°C. The digest ran overnight using gel electrophoresis at 15V on a 0.8% gel. The southern blot protocol was followed from “Current Protocols In Molecular Biology”. Nytran filter and wick from Shleicher&Schuell were used for transfer pyramid apparatus or blotting procedure. Primers were designed to amplify a 746 base pair region in the center of the \textit{recQ} gene to serve as a probe. The probe was labeled with deoxy-cytidine-5’-triphosphate α-32P ordered form MP Biomedicals. The NEBlot® Kit was used for a random priming reaction to label the probe. 25ng of template probe DNA was denatured by boiling in a water bath for 5 minutes, and then
immediately placed on ice for another 5 minutes. P^{32} dCTP is incorporated into the probe by a random-primed DNA polymerization using the DNA polymerase I-klenow fragment. The probe was then cleaned using the PCR Qiagen Cleanup Kit to remove unincorporated P^{32} dCTP. Some alterations were made to the protocol as the nitrocellulose filter was baked for 3 hours at 70ºC instead of 2 hours at 80ºC. Herring sperm was used instead of salmon sperm in the prehybridization solution. Prehybridization and hybridization were incubated at 40ºC, rotating overnight and placed in glass cylinders using a Hybaid rotating hybridization chamber by Labnet. After the filter was rinsed off, it was tapped to blotting paper, inverted over saran wrap, which was taped to the back of the blotting paper to secure and seal. In a dark room the X-ray film was placed over the filter and placed into a cassette. The cassette is then placed in a -80ºC freezer for 3 hours of exposure.

- **Protocol for Generating a ΔuvrD, ΔrecQ Double Mutant**

The strain CD571, a ΔuvrA and ΔuvrD mutant was used to create a ΔrecQ, ΔuvrD double mutant strain called SG8-2 derived from an SG8 strain. 1µg of CD571 genomic DNA was used to transform SG5 into a ΔuvrD, ΔrecQ double mutant. Transformants were plated on 3µg/mL chloramphenicol due to the drug cassette contained a Tn7-based transposon inserted within the uvrD gene. The inactivated uvrD gene including the transposon insertion is approximately 4,247 base pairs in size. The original SG8 strain contained the transposon insertion and lacked the recQ gene but did not appear to be a homozygous recessive strain. SG8 stock was inoculated and grown to stationary phase twice, once with Spectinomycin and Chloramphenicol, and once without any antibiotics. After cells reached stationary phase without antibiotics, these cells were plated on chloramphenicol plates, colonies were isolated, and frozen stocks were made of eight strains. Of these strains, SG8-1, SG8-2, and SG8-7 seemed to have homozygous recessive gene knockouts.
Table 4. List of primers used for verifying lack of *uvrD* gene and *recQ* gene in double mutants.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Tm*(°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UvrD1a</td>
<td>GACCTGCTCCAAGCCCTCAA</td>
<td>60</td>
</tr>
<tr>
<td>UvrD2</td>
<td>CATCACAAAGCGCAGGAACG</td>
<td>57</td>
</tr>
<tr>
<td>GPS Primer S</td>
<td>ATAATCCCTAAAAAACTCCATTTCACCCCT</td>
<td>58</td>
</tr>
<tr>
<td>GPS Primer N</td>
<td>ACTTTATGGCTATAGCTATTTTG</td>
<td>51</td>
</tr>
<tr>
<td>RecQ1</td>
<td>CCGCAGTTCCGTCTCCTTCTCTTT</td>
<td>59</td>
</tr>
<tr>
<td>RecQ2</td>
<td>AGCACCTTGTCGTTTCCGC</td>
<td>61</td>
</tr>
</tbody>
</table>

Both GPS primers bind to the opposite ends of the transposon and extend outward into
downstream from the start of the *uvrD* gene. Primer UvrD1a binds about 20 base pairs from the start of the *uvrD* gene. Primer UvrD2 is a reverse primer, binding about 1.4Kb into the gene.

UvrD1a and GPS primer N yield a PCR product of 800 base pairs, indicating that the transposon inserted about 820 base pairs downstream from the start of the gene. UvrD2 and GPS primer S yield a PCR product of around 750 base pairs, indicating the transposon inserted about 1423 base pairs from the end of the gene (Fig 2).

![Diagram of transposon and primers](image)

**Figure 2.** Transposon is depicted in center of diagram and Primer S and N extend away from the transposon and into the area of insertion.

Image is taken from New England Biolab’s GPS-M instruction manual.

- **Protocol Used for Ultraviolet Irradiation Mediated Damage**

  125mL flasks with 10mL TGY were inoculated with an overnight culture and grown to an optical density between 0.1 and 0.2. Sterile 60 x 15mm polystyrene Petri dishes by Falcon® were first labeled for the strain and dose to apply. 1mL of culture of each sample was removed and placed into each dish. A germicidal ultraviolet lamp placed approximately 5.5 inches away from the surface was used to irradiate the cultures. The dose rate was always adjusted to 10J/m² for each experiment. Appropriate safety measures including gloves, long sleeves, and face
masks were used during exposure experiments. Petri dishes had covers removed when timing started, and were roughly agitated by hand since the broth is ultraviolet opaque. Every sample was irradiated to the appropriate dose before dilutions were made. Samples were serially diluted in magnesium sulfate immediately after exposure, then spread onto plain TGY plates. This protocol was adapted from the paper by Kitayama, Asaka, and Totsuka (Kitayama et al., 1983).

- **Protocol Used for Hydrogen Peroxide Mediated Damage**

  Cells were grown to exponential phase as described previously, and separate flasks were used for each concentrated dose. Hydrogen peroxide mediated DNA damage was administered by concentrations of doses at 20mM, 40mM, and 60mM. 30% Hydrogen peroxide was used from Fisher Scientific. Hydrogen peroxide was added directly to the flasks after the exact volume of broth was determined. Flasks were then incubated for 1 hour at 30ºC with shaking at 200rpm. After 1 hour, cultures were immediately removed and diluted in 10mM magnesium sulfate then plated on TGY plates. Protocol was adapted from the paper by Wang and Schellhorn (Wang and Schellhorn, 1995).

- **Protocol Used for Mitomycin C Mediated Damage**

  Cultures were grown to exponential phase as previously described. Pre-exposure samples were taken from each flask, diluted and plated. Stocks of 500µg/mL were made from 2mg Powdered Mitomycin C containing 48mg NaCl from Acros Organics. A constant concentration of 20µg/mL of Mitomycin C was added to each flask and samples were obtained from flasks at 5, 10, 15, and 20 minutes. The proper concentration of mitomycin C was added, and then the flask was immediately placed in a shaking incubator at 30ºC. The same flask is removed slightly before each time point, and a sample is taken and placed in a microcentrifuge tube containing 10mM magnesium sulfate. The flask is quickly placed into the incubator, serial dilutions are
made, and samples are plated while waiting for the next time point. This protocol was adapted from Kitayama, Asaka, and Totsuka (Kitayama et al., 1983).

- **Protocol for Generating Plasmid SG27**

A plasmid was created to make a ΔrecJ mutant, however after multiple transformation attempts, it was discovered that the plasmid had a non-functional chloramphenicol drug cassette. pTNK101 was used to amplify the chloramphenicol cassette, and strain R1 was used to re-amplify the upstream and downstream regions using Vent polymerase (NEB). The plasmid was thus re-engineered using Vent instead of Taq polymerases. The construct was inserted into pBlueScript KS2, plasmid strain pDD769. The plasmid vector was first linearized using KpnI and PstI prior to transformation. New primers were designed to amplify the upstream region fused to the drug cassette the forward primer containing a KpnI restriction site on the 5’ end. The reverse primer was designed with complementarity to the chloramphenicol drug cassette, and has an EcoRI restriction site on the 5’ end of the primer. The 1Kb downstream region was amplified using a forward primer with an EcoRI restriction site on the 5’ end, and a reverse primer with a PstI site on the 5’ end. The 1.8Kb upstream region of the recJ gene and the chloramphenicol drug cassette were cloned into a PGEM®-T vector, and the construct was subsequently excised using EcoRI and KpnI. The downstream region was similarly excised from a PGEM®-T vector using EcoRI and PstI. Using these specific restriction endonucleases, the 3 pieces should ligate together according to the complementary overlapping sticky ends generated by the restriction enzymes. Ligations using T4 ligase (NEB) took place overnight and were at 16°C in an iCycler thermocycler (Bio-Rad). The plasmids were transformed into JM109 competent cells using the PGEM®-T Promega transformation kit. White colonies were picked.
and patched onto LB plates containing 34µg/mL chloramphenicol to ensure the drug cassette worked. Growing transformants were selected, and the plasmids were purified and underwent restriction endonuclease analysis to determine if the correct parts of the construct were present.

Table 5. Lists primers used for generating pSG27.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Tm*(°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RecJupRE</td>
<td>GTTACCGTCAGACCAACGCCGCTGTT</td>
<td>65</td>
</tr>
<tr>
<td>CatDownbRE</td>
<td>GAATTCTTAAGGGCACCAATAACTGC</td>
<td>57</td>
</tr>
<tr>
<td>RecJDownRE</td>
<td>GAATTCAGCGCTCGAGCGCCTGCT</td>
<td>67</td>
</tr>
<tr>
<td>RecJDown2RE</td>
<td>CTGCAGCGCCCCCAGAGATGCGGTGCC</td>
<td>70</td>
</tr>
</tbody>
</table>

- **Protocol Used for Determining Transformation Efficiencies**

  All transformations for transformation efficiency calculations were conducted using pSG27 and strains R1 and SG4 (ΔrecD). Plasmids were digested with endonuclease DraIII, and run on a gel to ensure DNA was linear. Circular plasmids were also used, along with a no DNA control to calculate efficiencies, and a total of 42 separate transformations were conducted. 486.055ng of undigested circular plasmid and 532.266ng of linear plasmid were used for individual transformations. Three separate flasks of R1 (called R1a, R1b, and R1c) were grown up to exponential phase, and the optical densities measured were respectively: 0.164, 0.101, and 0.160. Three separate flasks of the ΔrecD strain or SG4 (Da, Db, and Dc) were grown up to exponential phase, and the optical densities measured were respectively: 0.139, 0.149, and 0.144. 282µL of 1M CaCl was added to each flask of culture once cells were determined to have entered exponential phase (9.4mL left in each flask) to obtain a 30mM concentration. For each flask, three transformations were conducted for circular plasmids and for linear plasmids. Only one blank or mock transformation was conducted for each flask (Table 6)
Table 6. Depicts each separate transformation using individual strains inoculated separately and form of DNA used for each transformation.

<table>
<thead>
<tr>
<th>Flask</th>
<th>Linear DNA</th>
<th>Circular DNA</th>
<th>No DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1a</td>
<td>3 transformations/ tubes</td>
<td>3 transformations/ tubes</td>
<td>1 transformation/tube</td>
</tr>
<tr>
<td>R1b</td>
<td>3 transformations/ tubes</td>
<td>3 transformations/ tubes</td>
<td>1 transformation/tube</td>
</tr>
<tr>
<td>R1c</td>
<td>3 transformations/ tubes</td>
<td>3 transformations/ tubes</td>
<td>1 transformation/tube</td>
</tr>
<tr>
<td>Da</td>
<td>3 transformations/ tubes</td>
<td>3 transformations/ tubes</td>
<td>1 transformation/tube</td>
</tr>
<tr>
<td>Db</td>
<td>3 transformations/ tubes</td>
<td>3 transformations/ tubes</td>
<td>1 transformation/tube</td>
</tr>
<tr>
<td>Dc</td>
<td>3 transformations/ tubes</td>
<td>3 transformations/ tubes</td>
<td>1 transformation/tube</td>
</tr>
</tbody>
</table>

1mL of culture was placed into a plastic 25mL corning tube, along with 7µL of the DNA mixture (both linear and circular contained identical concentrations of NEB3, BSA, and elution buffer used to purify plasmids). Each individual transformation (tube) was plated on 3 TGY plates with 3µg/mL chloramphenicol, and colony forming units were counted. 50µL of the culture was directly placed onto each plate except for mock transformations (no DNA control) for those plates. Serial dilutions were made at $10^{-4}$, $10^{-5}$, and $10^{-6}$ with three plates for each dilution.
CHAPTER 2 RESULTS

2.1 The Construction and Characterization of the \( \Delta recD \) Strain SG4

In order to determine the importance of the protein RecD in repair processes, the gene was removed via gene replacement by homologous recombination using a construct containing 1kb of homologous sequence upstream and an 800 base pair sequence downstream of the gene with a hygromycin drug cassette inserted between the two regions. Transformants were screened on selective media containing hygromycin, and resistant strains were isolated. Genomic DNA was purified from each putative deletion, and PCR based analysis was used to verify the presence of the construct and to verify the presence of a gene knockout. The construct was confirmed using primers that amplified a 1,461 base pair fragment that included a portion of the hygromycin drug cassette and 300 base pair of genomic DNA downstream from this insert (Fig 4).

Figure 3. 1 Kb upstream and 800bp downstream region of \( recD \) gene are labeled in red. 1,119bp hygromycin drug cassette insert is orange rectangle in center. Locations of two primers used are indicated with blue arrows. Image is taken from Vector NTI Suite.
Figure 4. Verification of recD gene replacement by hygromycin resistance drug cassette. Lane 1 2-log DNA ladder (0.1-10kb), Lane 2, SG1, Lane 3, SG4, Lane 4 SG9, Lane 5, negative R1 control, Lane 6, no DNA control.

The size of the band is slightly smaller than the 1.5Kbp ladder marker, indicating that the construct has indeed inserted and has undergone recombination at the proper site. PCR analysis reveals the presence of the drug cassette, and the downstream region crossed over at the appropriate place within the genome. The fragments in lane 3 were generated from a strain designated SG4, the mutant strain used for all future studies of the ∆recD mutant. Amplification of this fragment was not visible when R1 genomic DNA was used. A second intense band appeared on the gel at approximately 0.4 Kbp, but it appears in all of the strains examined, indicating that these primers bind nonspecifically to at least one other site.

PCR analysis was also implemented to establish that SG4 was homozygous recessive for the ∆recD allele. The sensitivity of PCR allows detection of even a single copy of the recD gene within SG4’s polyploid genome. Primers were designed to amplify a 404 base pair region in the
center of the recD gene to determine if the gene is present in the mutant strains. Two R1 controls were used as a positive control and, three mutant strains were analyzed with the same primer (Fig. 5).

![Image](image.jpg)

**Figure 5.** Verification that SG4 is homozygous recessive for ΔrecD allele. Lane 1, 2-log ladder (0.1-10kb), Lanes 2-3, positive R1 control, Lane 4 SG1, Lane 5, SG4 Lane 6 SG9, Lane 7 no DNA control

The results from the gel indicate that allele is homozygous. No banding pattern is visible in the three mutant strains, as expected for a complete deletion of the gene. Strain SG4 is depicted in lane 5. Both R1 positive controls (lanes 2 and 3) show the expected recD bands, indicating that the primers amplify the recD gene.

- **The Role of RecD in the DNA Damage Tolerance of Deinococcus radiodurans**

(Ionizing Radiation)

Once strain SG4 was determined to be a homozygous ΔrecD mutant, further studies were conducted to determine if the recD gene product contributes to DNA damage repair processes in *D. radiodurans*. Fig. 6 depicts the effect that deletion of the recD gene has on the ionizing radiation resistance of this species. The putative 5’ to 3’ helicase activity RecD in wild type strain R1 does not appear to be necessary for ionizing radiation resistance. The only indication of loss of the RecD protein is observed at the highest dose administered where there is a slight
(1.5 fold) difference (unpaired student t-test, \( p=0.0006221378, \text{df}=22 \)) of means in survival when R1 and the \( \Delta \text{recD} \) mutant strain SG4 are compared. These results suggest that protein RecD may be involved in repair of ionizing radiation-mediated damage. The initial increase in colony forming units is most likely a consequence of the long exposure time needed to acquire the dose indicated. The rate of exposure is 1,080Gy per hour. The lengthy exposure time allows cell division, initiated before irradiation, to be completed causing a slight increase in cell numbers.

Figure 6. Ionizing radiation resistance of SG4. Each value is the mean of 3 experiments four replicates per experiment \( n=12 \) for SG4. Each value is the mean of six experiments, four replicates per experiment \( n=24 \) for R1

The error bars in the above graph show the standard deviation for the surviving fraction data points. The standard deviations are plotted in this fashion for all of the remaining graphs in this thesis.

(Ultraviolet Light)

SG4 was exposed to UV light generated by a germicidal lamp. As seen in Fig. 7, gene replacement by homologous recombination of \( \text{recD} \) had little effect on survival.
Strain SG4 exhibited the same shoulder resistance as wild type strain R1. There is no drop in viability until a dose of 400 J/m² was reached. The mutant strain was twofold more sensitive to UV light relative to R1 at the 1200 and 1600 J/m² doses, but this difference is not statistically significant (unpaired student t-test, p=0.6852260000, df=34).

(Mitomycin C)

Since strain SG4 had shown high resistance to other DNA damaging agents, mitomycin C was chosen to observe the role of protein RecD in repairing DNA interstrand cross-links. SG4 was challenged with constant exposure to mitomycin C at a concentration of 20µg/mL. At varying time intervals, aliquots of the cultures were serially diluted and plated onto TGY agar that did not contain mitomycin C. Survival was evaluated at time intervals beginning 5 minutes after exposure to mitomycin C. Mitomycin C exposure resulted in a rapid drop in viability (Fig. 8) in both the wild type and mutant ArecD strain.
Exposure to cross-linking agent, mitomycin C, yields a 1000-fold decrease in survival in the wild type strain after a 20 minute exposure. The lower mean survival of SG4 (5 fold relative to R1) observed after 20 minutes is statistically significant (unpaired student t-test, p=0.0103162990, df=16), otherwise the survival of R1 and SG4 are identical. The RecD protein does not appear to be necessary for repair of DNA cross-links caused by the effects of mitomycin C.

2.2 The Construction and Characterization of the \textit{ArecQ} Strain SG5

In order to characterize the role of helicase RecQ in DNA repair processes, the gene was replaced with a gene conferring spectinomycin resistance using gene replacement by homologous recombination as previously described. Putative \textit{ArecQ} transformants were selected on media containing spectinomycin, and PCR based analysis was used to verify whether the resistance gene was present and the \textit{recQ} gene absent.
Figure 9. Diagram of the recQ replacement construct engineered depicting where primers bind to upstream region of recQ gene, and the location within the spectinomycin/streptomycin drug cassette. Image created with Vector NTI software.

Primers were made to amplify a 263bp portion of the drug cassette and a 237bp region immediately upstream region of the recQ gene (Fig. 9). The amplified fragment is 500 base pairs in length, and includes a portion of the drug cassette. These primers verified the presence of the construct within the transformed genome, and verified that recombination took place in the correct location.

Figure 10. Verification of recQ gene replacement by spectinomycin resistance drug cassette. Lane 1 2-log DNA ladder (0.1-10kb), Lane 2, negative R1 control, Lane 3, SG5, Lane 4 SG2, Lane 5, SG3 putative mutant strains, Lane 6, plasmid pino103-2 as positive control, Lane 7 no DNA control
The gel depicted (Fig. 10), indicates ample amplification of an appropriately sized fragment, and this band is only present in the putative mutant strains. Lane 3 was generated from a strain designated SG5; the mutant strain used for all future studies of the ∆recQ mutant. Amplification was specific for strains carrying the spectinomycin cassette. Strain R1 showed no evidence of amplification (lane 2). A plasmid carrying the construct used to construct SG5 served as the positive control (lane 6).

To establish that the strain was homozygous for the ∆recQ allele, an attempt was made to amplify recQ using primers that anneal upstream and downstream of the intact gene. The intact recQ gene yields produce a 2,475 base pair fragment.

![Figure 11](image)

**Figure 11.** Gel does not verify that SG5 is homozygous recessive for ∆recQ allele. Lane 1 2-log DNA ladder (0.1-10kb), Lane 2, positive R1 control, Lane 3, SG5, Lane 4 SG2, Lane 5 SG3 putative mutant strains, Lane 6 no DNA control

The results of this analysis (Fig. 11) were ambiguous and inconclusive. A band of approximately 2500bp appeared in the putative mutant strain, but it could be the result of nonspecific amplification; the strain R1 control (lane 2) contains several non-specific bands.
Repeated attempts were made to remove possible nonspecific banding pattern, however none were successful, so the Southern blot technique was employed in an attempt to resolve the ambiguity.

Primers RecQ1 and RecQ2 were used to amplify a 746 base pair region in the center of the \textit{recQ} gene to serve as a labeled probe. The probe detected the presence of the gene in the R1 strains for each separate digest, but there was no evidence of the gene in the strain SG5 as there are no bands present in the SG5 lanes (Fig 12).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{southern_blot.png}
\caption{Verification that SG5 is homozygous recessive for \textit{Delta}recQ allele using Southern Blot. \larenge 1, Ladder, \larenge 2, R1 genomic DNA digested by KpnI, \larenge 3, SG5 genomic DNA digested by KpnI, \larenge 4, R1 genomic DNA digested by ClaI, \larenge 5, SG5 genomic DNA digested by ClaI}
\end{figure}

The first two lanes are R1 and SG5 strains digested with restriction endonuclease KpnI producing a 5.6Kb fragment surrounding the \textit{recQ} gene. The last two lanes are R1 and SG5 strains digested with endonuclease ClaI, producing a 4.1Kb fragment surrounding the \textit{recQ} gene. The probe bound to the \textit{recQ} gene within restriction fragments produced by KpnI and ClaI in
both of the lanes containing wild type strain R1 genomic DNA. The dark spots are the locations where the radioactive labeled probe bound to the gene. The probe did not bind either digested SG5 lanes, confirming that strain SG5 is a homozygous mutant lacking the gene.

- The Role of RecQ in the DNA Damage Tolerance of *Deinococcus radiodurans* (Ionizing Radiation)

SG5 was not sensitive to the effects of ionizing radiation. As indicated in Fig. 13, there is no difference between R1 and SG5 mutant strain when the strains are exposed to ionizing radiation. Apparently, the 3’ to 5’ helicase apparently is not necessary to repair damage incurred by gamma ionizing radiation.

![Graph](image)

**Figure 13.** Ionizing radiation resistance of SG5. Each value is the mean of three experiments four replicates per experiment n=12 for SG5. Each value is the mean of six experiments, four replicates per experiment n=24 for R1 at 0Gy only

(Ultraviolet Light)

Ultraviolet light doses were administered to SG5 and compared to R1 to determine the importance of RecQ helicase in repairing pyrimidine dimers. There is no difference in between these strains viability the UV dose exceeds 1200J/m² (Fig. 15).
Figure 14. UV radiation resistance of SG5. Each value is the mean of six experiments three replicates per experiment n=18 for SG5. Each value is the mean of 9 experiments three replicates per experiment n=27 for R1.

(Mitomycin C)

Mitomycin C was used to observe the effects of RecQ on repairing cross-linked DNA. SG5 was challenged with constant exposure to mitomycin C at a concentration of 20µg/mL. Exposure to cross-linking agents such as mitomycin C does not significantly decrease the surviving fraction of the ∆recQ mutant as compared to R1 (Fig. 15).
Figure 15. Mitomycin C resistance of SG5. Each value is the mean of three experiments, three replicates per experiment n=9

The last time interval measured approximately yields a 5-fold decrease in survival compared to the wild type strain.

Hydrogen peroxide mediated damage was chosen to further characterize the role of the helicase since it did not appear sensitive to any prior challenges. Lastly the single mutant was exposed to the oxidizing agent hydrogen peroxide to determine the effects on survival. R1 and SG5 strains were exposed to 20mM, 40mM and 60mM concentrations of hydrogen peroxide constantly for one hour before cultures were serially diluted and plated on plain TGY media.
After the first exposure, the surviving fraction of SG5 immediately decreases as compared to R1. The largest difference in survival observed is a ten-fold decrease at both 40mM and 60mM concentrations. At a concentration of 20mM hydrogen peroxide, there is statistically significant difference found when comparing R1 to SG5 (unpaired student t-test, p=0.0407191298, df=19). All other values have significance indicating that helicase RecQ is important for repairing oxygen radical mediated DNA damage.

2.3 The Construction and Characterization of the *ArecO*, *UvrD* Strain SG8-2

In order to determine whether there was a functional overlap between RecQ and UvrD (helicase II); both of which are 3’ to 5’ helicas, a double mutant strain was constructed.
Genomic DNA was purified from CD571 a \textit{uvrD:}\textit{TnDrCat}, \textit{AuvrA} strain created by Chris Davis Guillot (unpublished data) and one \(\mu\)g of DNA from this strain was used to transform strain SG5 to a \textit{uvrD::TnDrCat}, \textit{ArecQ} strain. CD571 carries a 4,247 base pair Tn7-like transposon inserted into the \textit{uvrD} gene, rendering its gene product nonfunctional.

PCR amplification was used to verify the \textit{recQ} disruption in the double mutant strains. The primers used in the gel below were designed to amplify a 746 base pair portion in the center of the \textit{recQ} gene. The gel (Fig. 17) verifies the presence of the gene in R1.

![Image of gel](image.png)

\textbf{Figure 17.} Verification that putative mutant strains are homozygous recessive for \textit{ArecQ} allele. Lane 1, 2-log DNA ladder (0.1-10kb), Lane 2, R1, Lane 3, CD571, Lane 4, SG8-1, Lane 5 SG8-2, Lane 6, SG8-7, Lane 7 no DNA control

Primers were designed to amplify a small 473 base pair portion near the center of the \textit{uvrD} gene, which surrounds the transposon insertion. The size of \textit{uvrD} gene without an insertion is 473 base pairs. If the gene is present in an uninterrupted copy, the primers should amplify a 473 base pair portion; and if the transposon is present a 4,720 base pair region will be amplified.
Figure 18. Verification of transposon insertion in putative mutant strains. Lane 1, 2-log DNA ladder (0.1-10kb), Lane 2, R1, Lane 3, CD571, Lane 4, SG8-1, Lane 5, SG8-2, Lane 6, SG8-7, Lane 7, no DNA control. Bright bands at bottom of gel are primer dimers.

The gel above (Fig. 18) shows evidence that the transposon insertion is present in all mutant strains, including CD571 in lane 3, and the wild type portion of $uvrD$ gene is found in R1 (lane 2) but not the other strains. There are nonspecific amplification bands but they are very faint and are identical to those exhibited in the original CD571 mutant strain while R1 displays very high amplification of the 473 base pair product of the $uvrD$ gene.

- **The Role of UvrD and RecQ in the DNA Damage Tolerance of Deinococcus radiodurans**

Once the strain was verified to be a $\Delta recQ$, $\Delta uvrD$ double mutant, it was termed strain SG8-2 and further studies were conducted to characterize the double mutant strain. The first study conducted was the comparison ionizing radiation resistance of strain CD571 with strain SG8-2, since the $recQ$ gene has already been analyzed and determined to yield a radiation resistant strain.
Figure 19. Ionizing radiation resistance of CD571, NS113 and SG8-2. Each value is the mean of three experiments three replicates per experiment n=9

The highest dose applied to strain SG8-2 was 5,400Gy, and no further data was collected beyond that dosage. Data from the other strains were collected by Chris Davis Guillot. Strain CD571 and R1 have the same shoulder resistance from 0 to 1,000Gy absorbed dose, differing from the double mutant strain. Strain NS113 (ΔuvrD), the single helicase mutant exhibits the same resistance as R1, which is similar to ΔrecQ and ΔrecD mutants. The largest difference in survival between strains NS113 and SG8-2 is a 7-fold decrease at 5,400Gy, indicating that there is some overlapping activity between the RecQ and UvrD helicases.
Ultraviolet radiation survival was the next DNA mutagen examined. The single $\Delta$recQ mutant, strain SG5 is not sensitive to ultraviolet irradiation (Fig. 14). Overall, there are no significant differences between R1 and SG8-2 when ultraviolet irradiation is applied (unpaired student t-test, $p=0.7820207436$, df=49).

Lastly, mitomycin C was used to observe the importance of genes $uvrD$ and $recQ$ in the repair of intrastrand crosslinks. Differences were observed between the single mutant strain SG5 (Fig. 15) and strain CD571 versus the double mutant strain SG8-2 (Fig. 20). After 10 minutes of exposure, there were no countable plates for strain SG8-2.
CD571 and SG8-2 lack components of the UvrABC excision repair pathway which is required for the repair of cross-linked DNA. As indicated, strains CD571, and SG8-2 are 100 fold more sensitive than R1 for both time exposures at 5 and 10 minutes, indicating that UvrD is necessary for the repair of mitomycin C mediated damage. Strain SG8-2 is 1,500X more sensitive after 5 minutes and is 1,200X more sensitive than R1 at 10 minutes. No growth was detected on plates exposed after 10 minutes. These results are statistically significant (unpaired student t-test, p=0.0000001242, df=16) indicating that both helicases are very important for repairing cross-linked DNA.

2.4 Transformation Efficiencies Using ΔrecD Strain SG4

Plasmid SG27 was generated for the purpose of deleting the gene recJ by gene replacement, and subsequently to create a ΔrecD ΔrecJ double mutant strain. A chloramphenicol drug cassette was inserted between the sequence corresponding to 1kb
upstream and downstream of the *recJ* gene of *D. radiodurans* R1. In order to verify the construct, the plasmid was digested with endonucleases EcoRI and KpnI which excise a 1.8kb upstream region fused to the chloramphenicol drug cassette, and EcoRI and PstI which excise the 1kb downstream region (Fig. 22).

The results (Fig. 22) indicate that all plasmids in lanes 2-11 have the upstream region and chloramphenicol drug cassette. Lanes 13-22 have a band the size of the 1kb marker in the ladder indicating that the 1kb downstream fragment is also present within the plasmid. All plasmids have the proper construct present, including pSG27 which is used for all transformation experiments.

**Figure 22.** Verification of construct present in all plasmid strains isolated. Lanes 1 and 12 are 2-log DNA ladder (0.1-10kb), Lane 2, pSG21, Lane 3, pSG22, Lane 4, pSG23, lane 5, pSG24, lane 6, pSG25, lane 7, pSG26, Lane 8, pSG27, Lane 9, pSG28, Lane 10, pSG29, Lane 11, pSG30, Lanes 2-11 are putative plasmid constructs digested with EcoRI and KpnI, Lane 12, 2-log DNA ladder (0.1-10kb), Lane 13, pSG21, Lane 14, pSG22, Lane 15, pSG23, Lane 16, pSG24, Lane 17, pSG25, Lane 18, pSG26, Lane 19, pSG27, Lane 20, pSG28, Lane 21, pSG29, Lane 22, pSG30, Lanes 13-22 are putative plasmid constructs digested with EcoRI and PstI
When transformations were conducted to obtain \(\Delta recJ\) single mutants and \(\Delta recD, \Delta recJ\) double mutants, it appeared that \(\Delta recD\) strain SG4 incorporated linearized DNA with greater efficiency. Strains SG4 and R1 were grown under identical conditions in 10mL TGY until they reached early-log phase (optical density of at least 0.1), and cultures were transformed with an equivalent amount of linearized plasmid. Transformation of SG4 yielded between 68-98 chloramphenicol-resistant colonies when 30\(\mu\)L of undiluted culture was plated, whereas wild type strain R1 only produced 4-5 colonies for the same amount, suggesting that protein RecD was limiting transformation efficiency.

As in \(E. coli\), it appears that the deletion of the \(recD\) gene in \(Deinococcus\) yields highly transformable strains without the need for complicated chemical treatments (Russell et al., 1989; Courcelle et al.). The transformation of linear and circular plasmids (pSG27) was positively affected by removal of the \(recD\) gene (Table 7). On average there was an approximately sixteen-fold increase in transformation efficiency in the mutant \(\Delta recD\) strain when 500\(ng\) of DNA was used in the transformation protocol.

**Table 7.** Increased efficiency of natural transformation in SG4 \((recD)\). Values are the average of 42 separate transformation experiments.

<table>
<thead>
<tr>
<th>pSG27 DNA Type</th>
<th>Strain</th>
<th>Strain Average Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circular</td>
<td>R1</td>
<td>25 cfu/(\mu)g DNA</td>
</tr>
<tr>
<td>Linear</td>
<td></td>
<td>14 cfu/(\mu)g DNA</td>
</tr>
<tr>
<td>Circular</td>
<td>SG4 ((recD))</td>
<td>454 cfu/(\mu)g DNA</td>
</tr>
<tr>
<td>Linear</td>
<td></td>
<td>201 cfu/(\mu)g DNA</td>
</tr>
</tbody>
</table>

2.5 Results Summary

Protiens RecQ and RecD individually are not essential for ionizing radiation, ultraviolet radiation, or mitomycin C resistance. Hydrogen peroxide yielded interesting results for strain SG5 \((\Delta recQ)\) indicating the 3’ to 5’ helicase may play an important role the repair of the oxidative damage generated by this mutagen. Strain SG4 yielded interesting results when
transformation efficiencies were compared with wild type, and similar results were observed with *E. coli* Δ*recD* mutants (Russell *et al.* 1989). Recombination rates greatly increased, presumably due to the lack of exonuclease activity necessary to degrade foreign DNA due to the anti-recombinational activity of protein RecD.

Both single and double Δ*uvrD* mutants are highly sensitive to mitomycin C. It is known that a single *uvrD* mutant is insensitive to ultraviolet and ionizing radiation (Chris Davis Guillot, unpublished data). Strain SG8-2 consistently has lower survival levels in most experiments conducted. These mutants lack two 3’ to 5’ helicases, and are defective in RecFOR repair, base excision repair (targets alkylation, and base mismatches), nucleotide excision repair (targets cross-links, and pyrimidine dimers), and the mismatch repair system due to lack of both RecQ and UvrD helicases. These mutants are more sensitive to all DNA damaging agents, and there is a significant decrease in resistance, especially visible with mitomycin C and ionizing radiation exposure.

### 2.6 Discussion

Removal of the *uvrD* and *recQ* genes in *Deinococcus radiodurans* resulted in a tenfold decrease in survival. It is uncertain how these proteins fit into unique models of DNA repair. In light of these results, it is thought that there must be other systems involved in DNA damage repair. The results in the study presented indicate that Δ*recQ* strain SG5 is insensitive to ionizing radiation, ultraviolet radiation, and mitomycin C exposure as there is little difference between the mutant and wild type strain R1 survival in all trials. This could be due in part to a redundant helicase functioning in the absence of helicase RecQ to unwind the strands for homologous recombinational repair. RecQ 3’-5’ helicase functions in conjunction with RecFOR and RecJ repair pathways. When RecQ helicase is removed from the complex, it is thought that another 3’-5’ helicase, UvrD may function in its place.
Recently, there have been two separate accounts describing the effect of different mutagens on *Deinococcus radiodurans*Δ*recD* mutants with differing results. Insertional mutagenesis was the technique used by both studies to inactivate the *recD* gene along with a separate gene replacement (Servinsky and Julin 2007; Zhou *et al.*, 2007). The results found by Zhou and colleagues seem to parallel the results found in this study. The results found by Servinsky and Julin contradict the results found in this study, indicating the mutant strain is much more sensitive than the wild type strain.

In the first study a Δ*recD* mutant was created and compared to wild type strain R1 ATCC 13939 for its ability to survive exposure to various DNA damaging agents (Zhou *et al.*, 2007). The mutants were exposed to ionizing radiation and ultraviolet radiation. The results reported coincide with the results stated previously in this thesis. The Δ*recD* mutant was insensitive to exposure for neither damaging agent as compared to strain R1. Cells were exposed to ionizing radiation at room temperature while cells were growing in exponential phase. Ultraviolet irradiation was conducted after dilutions were plated onto TGY plates exposed to different doses. The mutant strain was found to be only slightly more sensitive to ultraviolet light than the wild type strain, again similar to the study in this thesis. The above two results from Zhou and colleagues bolster the results presented in this paper, indicating that the role of protein RecD is not necessary for ionizing radiation or UV resistance.

In contrast, the second study which compared the *recD* strain and BAA-816 provided evidence that inactivation of this gene leads to ionizing radiation sensitivity, ultraviolet radiation sensitivity, and no sensitivity to mitomycin C exposure (Servinsky and Julin, 2007). Since ATCC13939 and BAA-816 are not the same genotype, it is plausible that unknown polymorphisms found in these strains are responsible for the differences.
As reported previously (Servinsky and Julin, 2007), our ∆recD strain showed an increase in transformation efficiency using a slightly different protocol, suggesting that RecD is involved in the destruction of DNA as it enters the cells in a manner not unlike that described for RecD function in *E. coli*.

An interesting finding by Lovett and Sutera identified two helicases that together function to suppress a recombinational deficient ∆recJ mutant in *Escherichia coli* (Lovett and Sutera, 1995). This finding parallels the result found in *Deinococcal* ∆uvrD, ∆recQ mutants in that each single mutant is not sufficient to confer sensitivity to mutagens. UvrD and HelD are both helicases, which work in conjunction to prevent recombinational intermediates otherwise formed in the absence of exonuclease RecJ. Neither helicase mutation is sufficient to restore recombinational proficiency because the helicases either work together or have some overlapping activity. UvrD in *Deinococcus radiodurans* is thought to have the same activities as the identical protein in *E. coli*. Apparently UvrD in both *E. coli* and *D. radiodurans* contain overlapping activities, and this finding may give some valuable insight into future mutational analysis to pursue in *D. radiodurans*.

Strain SG5 was found to have sensitivity to hydrogen peroxide mediated damage which causes oxidation of bases, abasic sites, DNA-DNA intrastrand adducts, strand breaks, and DNA-protein crosslinks (Cadet *et al.*, 1999; Imlay and Linn, 1988). A ten fold decrease in survival is observed for 40mM and 60mM exposures to hydrogen peroxide as compared to wild type. Helicase RecQ is necessary for repairing hydroxyl radical mediated damage, and helicase UvrD may not be an efficient replacement helicase for the type of damage incurred.

Helicase UvrD is involved in mismatch repair and nucleotide excision repair in *Deinococcus* while helicase RecQ is involved in RecFOR mediated recombinational repair (Kim *et al.*, 2004; Makarova *et al.*, 2001; Killoran and Keck, 2006). Both 3’-5’ helicases bind
specifically to free 3’ ends of a double strand break. These helicases are thought to function in
the place of each other when cells undergo DNA damage repair. Strain NS113 is a \( \Delta uvrD \) strain,
and this strain was used as a control to compare the role of UvrD in repair with wild type strain
R1 (Chris Davis Guillot, unpublished), and the double helicase mutant strain SG8-2 after
exposure to ionizing radiation.

All helicase mutants show the same resistance as wild type, indicating that there must be
an alternative substitute helicase able to unwind DNA for repair. Strain NS113, SG4, and SG5,
all \( \Delta uvrD, ArecD, \) and \( ArecQ \) helicase mutants, respectively must have other compensatory
helicases in the genome. Ionizing radiation experiments yielded interesting results in that the
deletion of both helicases in the double mutant strain SG8-2 appears to have a cumulative effect
on surviving fractions because due to a 7 fold decrease in the double mutant as compared to the
single mutant strains. \( ArecQ \) strain SG5, and \( \Delta uvrD \) strain, NS113 display no sensitivity to
ionizing radiation; while strain CD571 has the same shoulder dose as strain R1 up to 1,620Gy it
is at least 20 fold less sensitive than SG8-2. Strain SG8-2 drastically decreases after the first
dose while strain CD571 gradually decreases. The difference in survival in strain SG8-2 is due
to the lack of overlapping activity shared by both 3’-5’ helicases.

Exposure to ultraviolet light had a minimal effect on all three single and double mutants.
SG5, a \( ArecQ \) mutant, displayed the least sensitivity to ultraviolet radiation as the surviving
fraction remained above wild type strain R1 for all doses. Helicase RecQ is not necessary for
repairing UV induced damage. Strain CD571 showed little sensitivity as the shoulder dose
paralleled strain R1 until a dose of 1,200J/m\(^2\) was reached. The results indicated that the \( \Delta uvrD, \)
\( \Delta uvrA \) double mutant strain is not sensitive to ultraviolet radiation until high exposures are
reached, so some form of dimer repair is still functional. Strain SG8-2 displayed the lowest
surviving fraction, for most doses where the largest decrease in survival was a 5 fold difference
at 800 and 1,200J/m$^2$. This indicates that both helicase deletions do have some effect on the repair of UV mediated DNA lesions.

Exposure to mitomycin C revealed significant differences between both single helicase mutant strains and the double helicase mutant strain. Strain SG5 was not sensitive to mitomycin C as compared to the wild type strain, and the RecQ protein was not needed to repair cross-linked DNA. Strain CD571 was sensitive to the mutagen and displayed an immediate decrease in survival after 5 minutes of exposure to 20µg/mL of mitomycin C. The $uvrD$ and $uvrA$ genes have importance in maintaining the genome after exposure to mitomycin C. The double mutant strain SG8-2 displayed an even greater decrease after a 5 minute exposure, suggesting a synergistic effect due to the deletion of both helicases. Strain CD571 decreased 100 fold as compared to R1 after 5 minutes, while strain SG8-2’s surviving fraction decreased 1,500 fold as compared to the wild type strain R1. There was a 15 fold difference between strains CD571 and SG8-2, and a 1,500 fold difference between strains SG5 and SG8-2 as the survival curve of SG5 closely paralleled the R1 curve. The difference in survival is due to the overlapping activities of both helicases. Helicase RecQ may be able to function in the UvrABC nucleotide excision repair pathway to unwind the damaged strand for repair, in addition to functioning in the RecFOR homologous repair pathway. The UvrD helicase also may be able to function in both repair pathways once the additional 3’-5’ helicase RecQ is rendered nonfunctional. It is uncertain how these proteins fit into unique models of DNA repair. Further studies are needed to elucidate how these two helicases function in the absence of the other.
REFERENCES


strains and the first discovery of insertion sequence element from deinobacteria. Gene 198:115-126.


VITA

Stephannie Gauthier Ruiz was born in April 1982 in Lafayette, Louisiana. She attended Saint Thomas More high school. Influenced by her mother’s interest in chemistry and microbiology, she majored in microbiology. Stephannie graduated from the University of Louisiana at Lafayette in 2004 with a bachelor of science in microbiology. She began her studies at Louisiana State University in July of 2005.