Investigating Survival Strategies of a Radioresistant Bacterium: Deinococcus Radiodurans

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Decoding the Mystery of the Most Radioresistant Life Form in the World: *Deinococcus Radiodurans*
Danielle Gerbic
Advisor: Matthew Lopper, Ph.D.

**Abstract**
When a cell’s DNA is damaged, replication proteins fall off of the replication fork and many cells die; however, some cells are able to use a replication restart process. *Deinococcus Radiodurans* is one bacterium that can utilize this replication restart process. Three replication proteins were synthesized and used to test if they were what allowed *D. rad* to use replication restart and reload the proteins onto the replication fork. Studies were unable to determine the precise purpose of these proteins in *D. rad*.

**Introduction**
During DNA replication a helicase separates the double stranded DNA into two single strands. This helicase is what creates a replication fork. Replication restart is able to “reload” the replication proteins back onto the fork. *D. rad* has been studied since the 50s and has been classified as the most radioresistant life form in the world. *D. rad* has proteins PriA, SSB, and DnaB that may be the reason it is able to withstand high amounts of radiation.

**Materials and Methods**
- Transformation and synthesis of replication proteins
  - PriA, SSB, and DnaB
  - Fast protein liquid chromatography
- DNA fork substrate construction
  - Labeled oligo DNA
- Helicase Assays
  - florescent polarization spectrophotometer
  - Polyacrylamide gels

**Results**

**DNA Fork Substrate**

<table>
<thead>
<tr>
<th>Partial Fork 2C</th>
<th>oML.211</th>
<th>25</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>oML.367</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

**Gel Result**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Lane Number</th>
<th>Amount Gels</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>DnaB</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SSB</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PriA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DnaB</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>ATP</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**FPLC Readout**

**Conclusions**
- Purifications produced pure proteins
  - 75%-95% purity
- DNA fork substrate was successfully created
- DnaB helicase failed to unwind duplex DNA
  - In both the helicase assays and gels

**Future Directions**
- Resynthesizing the replication proteins
  - Enzymatic activity could have been lost
- Studying protein-protein interactions
  - We studied protein-DNA interactions
- Finding another accessory factor for replication
- Perform helicase assays on an *E. coli* cell
  - *D. rad* could be the problem