 CHAPTER 6

A comparison of the recD mutant phenotypes of

P. syringae and E. coli

6.1 INTRODUCTION

The RecBCD complex is essential for recombination mediated repair of double strand breaks (DSBs) of DNA in E. coli (Smith, 1988). It was observed that mutations in recB and recC genes make E. coli sensitive to DNA damaging agents, like UV, ionizing radiation, and mitomycin C, due to the inability of the mutant strains to repair DSBs. These mutants have low cell viability even when grown at 37°C, presumably also due to the inability to repair DSBs that occur during normal growth. The RecD, the third component of the RecBCD complex, was however dispensable for the repair of DSBs in E. coli, as revealed by the normal level of resistance of recD mutant cells to DNA damaging agents. The recD mutants of E. coli, unlike recB and recC mutants, exhibit normal viability at 37°C, suggesting that recD probably does not have any important role in the repair process. However, the recD mutants of E. coli have some other interesting properties. The properties include (i) increased plasmid instability (Biek and Cohen, 1986), and formation of linear plasmid DNA (Cohen and Clark, 1986), (ii) changes in plasmid copy number (Seelke et al., 1987), and (iii) increase in adaptive mutation frequency (Foster and Rosche, 1999). The plasmid instability, increase of copy number and formation of linear plasmids in recD mutants was restricted to only certain class plasmids, and is not a general feature.

The recD mutants of E. coli were found to be sensitive to DNA damaging agents in combination with other mutations. For example, recD recJ double mutant (Lovett et al., 1988) and the recD recJ sbcB sbcCD quadruplet mutant were found to be sensitive to UV (Seigneur et al., 1999). Since our study indicated that the recD of P. syringae could complement the recD mutation of E. coli, with respect to λ-phage replication and T4 2' phage multiplication, it was interesting to compare other properties
between these two organisms. This chapter describes the results of this comparative analysis.

6.2 RESULTS

6.2.1 The *P. syringae* CS1 is relatively more sensitive to DNA damaging agents

To examine the susceptibility of *recD* mutant of *P. syringae* to DNA damaging agents, the sensitivity to UV and mitomycin was tested for CS1 and its isogenic parent R5 (Fig 6.1). Sensitivity to UV and mitomycin C was determined as described in Materials and Methods (Chapter 2). The results indicated that the *recD* mutant of *P. syringae* is moderately sensitive to both UV and mitomycin C. For example, in presence of 2 \( \mu g/ml \) mitomycin C the percentage survival of R5 and CS1 strains were 72% and 20%, respectively.

6.2.2 Plasmid instability of CS1

The *recD* mutations in *E. coli* are known to affect the copy number of plasmid (Biek and Cohen, 1986). To examine whether this holds true for the psychrotrophic *P. syringae*, the stability of the plasmids were determined in *P. syringae recD* mutants. The stability of two broad host range plasmids pUFR034 (IncW replicon) and pGL10 (IncP replicon) were tested.

The IncW plasmid pUFR034 that was found to have a low stability in the R5 strain, exhibited further instability in the *recD* disrupted mutant CS1. For example, only about 10 and 3.5% of the R5 and CS1 cells, respectively, retained the pUFR034 (Km') following their growth on nonselective medium for 24 hrs (Table 6.1). On the other hand, pGL10 was very stable in both R5, and CS1 (Fig 6.2). To exclude the possibility that the stability of pGL10 in CS1 is due to the presence of 53% intact *recD*, that might produce a truncated protein, the stability of pGL10 was tested in LDD22 (retaining only 31% of intact *recD*) and WT strains. The LDD22 strain did not lose the kanamycin resistance marker of pGL10 even up to 100 hours of growth on nonselective medium (data not shown).
Fig. 6.1 The recD mutant of *P. syringae* is modestly sensitive to DNA damaging agents UV (A) and mitomycin C (B). CS1, *recD* mutant of *P. syringae*; R5, parental strain of CS1. The sensitivity was calculated based on the c.f.u. of bacterial cells following their treatment to the agents as described under Materials and Methods (Chapter 2)
Table 6.1 The plasmid pUFR034 is more unstable in CS1

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>R5(pUFR034)</th>
<th>CS1(pUFR034)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>18.6</td>
<td>13.4</td>
</tr>
<tr>
<td>24</td>
<td>10.34</td>
<td>3.44</td>
</tr>
</tbody>
</table>

Fig. 6.2 Stability of plasmid pGL10 in *P. syringae*. CS1, recD mutant of *P. syringae*; R5, parental strain of CS1
6.2.3 *E. coli* recD gene does not complement the cold sensitivity of CS1

Having found that the *recD* gene of *P. syringae* complements the *recD* mutation in the λ phage plaque size assay, the ability of *E. coli* recD to complement the cold sensitivity of CS1 mutant was tested. The plasmid pKB65, which contained the *E. coli* *recD* on the broad-host-range cloning vector pGL10 was mobilized into CS1 by conjugation, and the Tc' Km' transconjugants were selected. The pGL10 without any insert was also mobilized to CS1 for use as an empty vector control. As shown in Figure 6.3 the *E. coli* recD failed to restore the growth of CS1 at 4°C. To rule out the possibility that the lack of complementation is due to the lack of expression of *E. coli* RecD in *P. syringae*, Western blot analysis was performed using a monoclonal antibody against the *E. coli* RecD (Amundsen et al., 2000). The Western analysis results indicated that the *E. coli* RecD protein is produced in *P. syringae* at both 22°C and 4°C (Fig 6.4). This suggested that the inability of *E. coli* recD to complement the growth of *P. syringae* at 4°C is not due to lack of expression of the protein, but may be due to the lack of functional domain of RecD, which is required for growth of at low temperature.

6.2.4 *E. coli* recD mutants are not cold sensitive

In order to examine whether *E. coli* recD mutants are sensitive for growth at low temperature (e.g. 10-15°C), growth profiles of the *E. coli* recD mutant CAG12135 (MC4100 *recD*1901::Tn10) at 37°C and 15°C were compared with that of isogenic parent MC4100 (Fig. 6.5). As shown in the Figure both strains grew at comparable rates at both the temperatures, suggesting that *recD* mutants of *E. coli* are not cold sensitive. Studies by Nichols et al. (1998) revealed that the Tn10 insertion in CAG12135 has occurred immediately after the 1542th nucleotide of 1824 nucleotides long ORF of *recD*, leaving 84% of the gene intact. Since the LDD11 strain of *P. syringae* that had 83% of the intact *recD* gene was not cold sensitive, the proficiency of *E. coli* CAG12135 to grow at low temperature could simply be due to the residual 84% intact *recD* in this strain. Therefore, another *E. coli* strain with a mutation at the N-terminal end was tested for its growth at low temperature. The growth profiles of the mutant V222, and its isogenic parent V66, at 37°C and 15°C were compared. The V222 strain had a C to T mutation in the 10th nucleotide of *recD* gene, thus changing the 4th codon.
Fig. 6.3 The *E. coli* recD gene does not complement the cold sensitivity of CS1. Growth profiles of R5(pGL10), CS1(pGL10) and CS1(pKB65) in AB broth at 4°C have been shown. pGL10, cloning vector alone; pKB65, plasmid containing the *E. coli* recD.
Fig. 6.4 *E. coli* RecD is expressed in *P. syringae*. The whole cell extracts of various strains were separated by SDS-PAGE on a 7.5% gel and was subjected to western blot analysis using anti RecD monoclonal antibody. MG1655recD::Tn10, recD mutant of *E. coli*; CS1, cold sensitive mutant; R5, parental strain of CS1; pKB65, plasmid containing the *E. coli* recD; pGL10, broad host range cloning vector.
Fig. 6.5 *E. Coli* with C-terminal disruption of RecD is not cold sensitive. Growth profiles of *E. coli* wild type strain MC4100 and the recD mutant MC4100 recD1901::Tn10 in LB broth at 37°C (A) and 15°C (B) have been shown.
Fig. 6.6 *E. Coli* with N-terminal disruption of RecD is not cold sensitive. Growth profiles of *E. coli* wild type strain (V66) and the recD mutant (V222) in LB broth at 37°C (A) and 15°C (B) are shown.
encoding arginine to TAA stop codon. It was observed that the V222 strain also was not cold sensitive, but exhibited slightly slower growth compared to parental strain V66, at both 37 and 15°C (Fig. 6.6). These results confirmed that the recD mutants of E. coli are not cold sensitive.

6.3 DISCUSSION

The study reported in this chapter was carried out for comparison of recD functions in psychrotrophic P. syringae and mesophilic E. coli. The results indicated that P. syringae recD mutant CS1, is slightly more sensitive to DNA damaging agents UV and mitomycin C. This is in contrast to the phenotype exhibited by the recD mutant of E. coli, which are resistant to UV and mitomycin C (Smith, 1988). Since the RecBCD pathway of homologous recombination plays important role in the repair of DSBs caused by UV, it is possible that the P. syringae RecD might have a unique role in the repair of DNA.

The recD gene of E. coli could not complement the cold sensitivity of CS1 even though the E. coli RecD is expressed in P. syringae as indicated by Western blot analysis. Therefore it is tempting to speculate that the P. syringae RecD is functionally different from its E. coli counterpart. However, possibilities like the inability of the E.coli RecD to form complex with the RecB and RecC proteins of P. syringae, or its improper folding in CS1 at low temperature have to be excluded before attributing a low temperature specific functional role for P. syringae RecD.

The growth of E. coli strains with mutation at N-terminal end (e.g. V222) and C-terminal end (e.g. CAG 12135) at low temperature (15°C) imply that the recD gene is not essential for low temperature growth in the mesophilic E. coli.