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Nucleotide excision repair is important for survival of hydrogen peroxide mediated killing in *Escherichia coli*

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Abstract: ***Escherichia coli*** **(*E. coli*) exposed to 1-3mM Hydrogen peroxide (H_2O_2) undergo killing which is induced by DNA damage. Oxidative stress mediated DNA damage can be repaired via DNA repair pathways like base excision repair (BER) and homologous recombination repair. In this study, we have investigated the role of nucleotide excision repair (NER) in survival to oxidative killing. *E. coli uvr*A mutant showed higher sensitivity to killing by H_2O_2. Exposure to 3mM H_2O_2 for 90 minutes led to 900-fold higher killing in *uvrA* compared to the wild type strain. The sensitivity of *uvrA* strain was observed across different conditions such as minimal nutrient condition and low aeration. Availability of nutrients and extent of aeration during H_2O_2 challenge both affected the magnitude of sensitivity but did not alter the sensitivity pattern. This study provides evidence that NER plays an important role in the survival of *E. coli* under high level of oxidative stress.**

Keywords: ***Escherichia coli,*** **H_2O_2, Nucleotide Excision Repair, oxidative stress, *uvr*A.**

1. INTRODUCTION

Oxygen is essential for sustenance of life in aerobically growing organisms; however, this oxygen itself acts as double edged sword. Aerobically growing organisms constantly generate reactive oxygen species (ROS) as by-products in the process of formation of water from oxygen. Nucleic acids, proteins, lipids and cell membrane components are potential targets for ROS which eventually lead to cell death [1], [2], [3]. Oxidative attack by H_2O_2 results in the production of modified bases such as 8-oxoG, Fapy-G in the DNA; of which 8-oxoG on incorporation in the DNA brings about the transition/transversion mutation [4], [5].

In *E. coli*, exposure to H_2O_2 at a concentration ranging from 1-3mM results in mode 1 killing which is caused by extensive damage to DNA. Similarly, exposure to higher concentrations of H_2O_2 causes mode 2 killing which is caused due to accumulation of non-specific damages [6]. Oxidatively damaged DNA can be repaired by well characterised DNA repair pathways in the cells such as base excision repair (BER) and homologous recombination repair. Nucleotide excision repair (NER) in *E. coli* is mediated by UvrABC endonuclease, a complex of UvrA, UvrB and UvrC proteins. This complex is responsible for removal of the DNA lesions that arise due to distortions in phosphodiester back bone [7]. Though the role of NER in repair of UV damage is well accepted, its involvement in repair of oxidative damage to DNA has been ambiguous. In order to investigate the role of NER in repair of DNA damaged due to oxidative agents we studied the survival of wild type and *uvrA* mutant of *E. coli* exposed to H_2O_2 under different conditions such as varying nutrient levels or varying aeration. Our results show that for all the conditions tested NER is required for optimal survival of the cells under oxidative stress.

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2. MATERIALS AND METHODS

Bacterial Strains, Media, Antibiotics and Chemicals

E. coli wild type and *uvrA* mutant strains are described in Table 1. The strains were obtained from Keio collection [8]. The strains were routinely grown in Luria Bertani medium (supplemented with 1.7 % Agar for solid medium). Minimal media composed of M9 salts (1x), 0.2% glucose, 0.002M $MgSO_4$ and 0.0001M $CaCl_2$. The medium was supplemented with 30µg ml^{-1} of kanamycin when required. LB agar was procured from the BD Difco and/or Hi-Media labs, India. Kanamycin was purchased from Sigma-Aldrich. H_2O_2 was procured as a 30% solution from Merck. M9 salts were procured from Hi-Media and chemicals like Glucose and Magnesium sulphate were purchased from Molychem and Calcium chloride was procured from SDFCL.

Table 1: Bacterial strains used in this study

E. coli K -12 Strain	Gene Mutation	Genotype	Source
BW25113	Wild type	Δ(*araD-araB*)*567*, Δ*lacZ4787*(::rrnB-3), λ-, *rph-1*, Δ(*rhaD-rhaB*)*568*, *hsdR514*	[8]
JW4019	*uvr*A	Δ*uvrA753*::kan, BW25113	[8]

H_2O_2 survival assays

Overnight grown cultures were inoculated in fresh LB medium at the rate of 1:100 and grown at 37° C with aeration (160 rpm shaking). When the cell density reached 1.8-2 x 10^8 cfu ml^{-1} (cfu: colony forming units), H_2O_2 was added to final concentration of 2mM or 3mM as required. After addition of H_2O_2 the cells were incubated in the dark at 37°C with 160 rpm shaking for 60 and 90 min respectively. Control cells not exposed to H_2O_2 were treated similarly. Cells were then serially diluted in sterile saline (0.85% NaCl in water) and plated on to LB agar plates and cfu counts were determined after 16-18 h of incubation at 37°C. The percentage survival was evaluated by comparing treated versus untreated cells.

To study the effect of aeration the survival assay was performed as described with the exception that the cells were incubated in the dark at 37°C without shaking i.e. static condition after addition of H_2O_2.

To study the effect of nutrition, overnight grown cultures in the LB medium were diluted in fresh M9 medium (1:50) with or without kanamycin 30µgml^{-1} and grown at 37°C with 160 rpm shaking. Survival assay was performed as described above when the cell density reached 1.8-2 x 10^8 cfu ml^{-1}.

3. RESULTS AND DISCUSSION

Previous studies in *E. coli* have given conflicting reports on the involvement of NER in the repair of oxidative damage to DNA. As NER mutants did not show sensitivity to killing by H_2O_2, in an earlier study, it has been hypothesized that in *E. coli* NER is not involved in the repair of DNA lesions caused by oxidative damage [9]. However, there have been indirect evidences suggesting the role of NER. For instance, elevated SOS response observed in *uvr*A mutant exposed to oxidative stress indicated the involvement of the nucleotide excision repair pathway in protecting cells against oxidative stress [10]. In order to clarify the role of NER in survival of H_2O_2 mediated damage to DNA, we studied effect of two different concentrations i.e. 2mM and 3mM of H_2O_2 on survival of wild type and isogenic *uvr*A mutant grown in rich medium (LB) (Fig. 1). The wild type cells showed about 24 % survival when exposed to 2mM H_2O_2 for 60 min. For the same treatment *uvrA* showed 5.8 % survival. After 90 min of exposure to same concentration of H_2O_2 the survival of wild type and *uvr*A was about 20% and 2.5% respectively. Under the above conditions *uvr*A cells exhibited moderately higher sensitivity compared to the wild type cells (Fig. 1). At an exposure for 60 min to 3mM H_2O_2, a similar trend of sensitivity was observed (Fig. 2). However, a remarkable increase in sensitivity of *uvr*A cells was seen after 90 min of exposure to 3mM H_2O_2. The difference in sensitivity over wild type was more than 900-fold (Fig. 2). This time dependent decrease in survival of *uvr*A cells suggests that NER pathway is important for the repair of DNA damage caused by oxidative stress in rich medium. In contrast to our results, Imlay and Linn (1987) did not observe sensitivity in *uvrA* strain upon H_2O_2 exposure for 15 min. One possible reason for this difference could be the longer H_2O_2 exposure time employed in our study (60 min and 90 min). Further we have used a complete deletion of *uvrA* while a *uvrA6* allele has been used earlier. *uvrA6* allele is a frameshift at positions 207-209 which is assumed to inactivate the protein [11].

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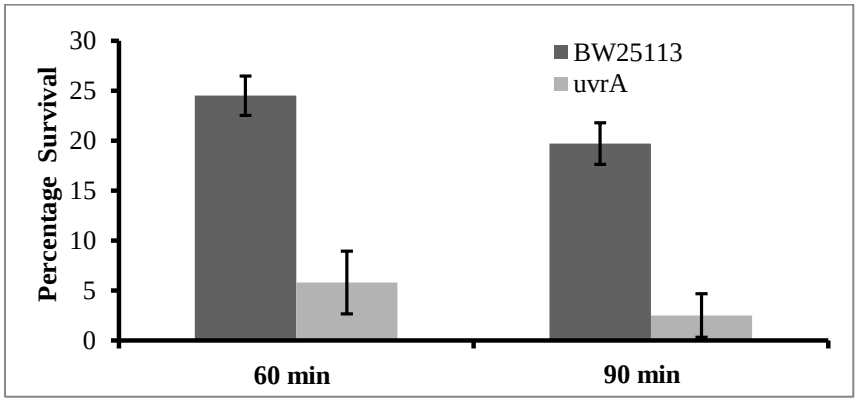

Fig. 1 Survival of *E. coli* exposed to 2 mM H_2O_2 in rich medium. Error bars represent SD of mean.

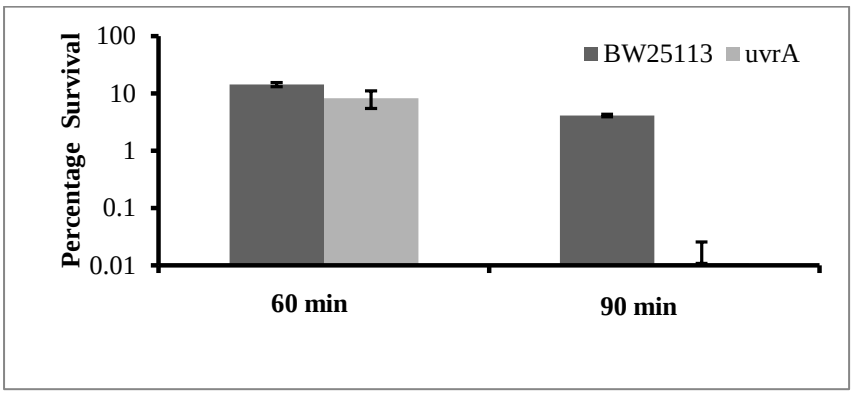

Fig. 2 Survival of *E. coli* exposed to 3 mM H_2O_2 in rich medium. Error bars represent SD of mean.

As under natural conditions most of the times bacterial cells experience nutrient deprivation, the effect of minimal medium on survival of H_2O_2 exposure was investigated. *E. coli* cells were grown in minimal medium and exposed to 3mM of H_2O_2 for 60 min and 90 min respectively. Both wild type *E. coli* and *uvr*A strain showed an increase in the percentage survival when compared with the rich medium (Fig. 3). Interestingly, *uvr*A mutant showed increased sensitivity compared to the wild type after 60 min H_2O_2 challenge as well as 90 min of H_2O_2 challenge. These results are consistent with what was reported in a previous study involving wild type *E. coli* K-12 [12]. In that study increased resistance to 15mM H_2O_2 exposure for different time periods was reported when the *E. coli* culture was grown at 29°C in M9 medium containing 0.3% Glucose; with 4 h exposure providing maximal protection. In the present study, overnight grown cultures were diluted in minimum media containing 0.2% Glucose and allowed to reach the density of 1.8-2 x 10^8 cfu ml^{-1} before exposure to H_2O_2. These results suggest that as with rich medium, even in minimal nutrient conditions NER has an important role in survival of H_2O_2 killing.

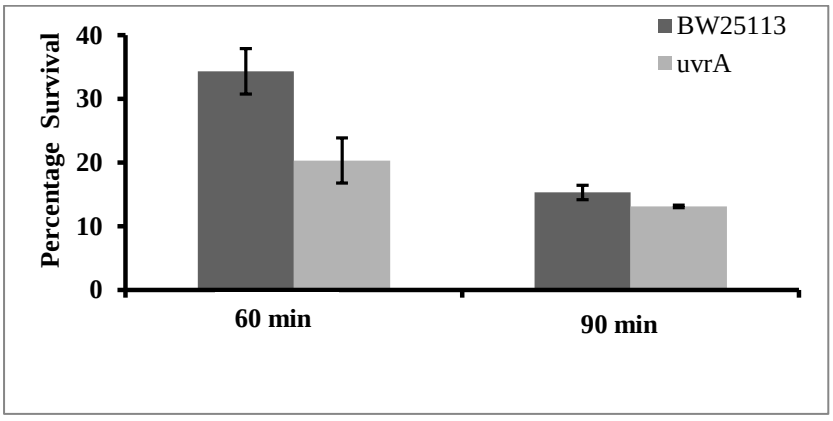

Fig. 3 Survival of *E. coli* exposed to 3 mM H_2O_2 in minimal medium. Error bars represent SD of mean.

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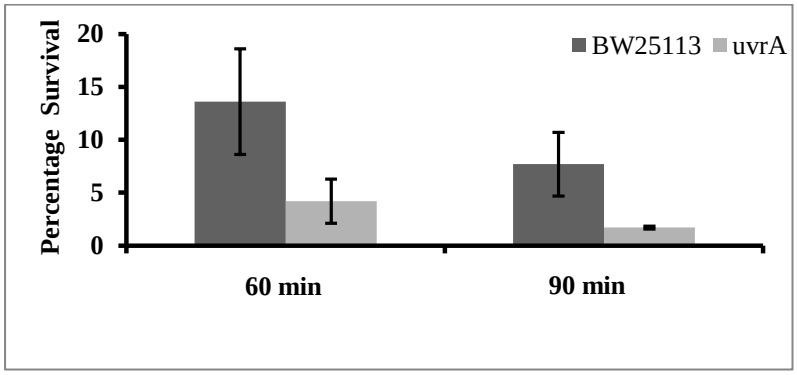

Fig. 4 Survival of *E. coli* exposed to 3 mM H_2O_2 in low aeration condition. Error bars represent SD of mean.

To study the role of aeration, *E. coli* and *uvr*A strains were exposed to oxidative stress in the low aeration i.e. static conditions for 60 min and 90 min respectively. Here too *uvr*A cells showed increased sensitivity over wild type cells (Fig. 4). In contrast to what was observed with optimal aeration conditions, *uvrA* cells which were hyper-sensitive after 90 min of exposure to 3mM H_2O_2 under aeration showed 170-fold improved survival with less aeration (Fig. 2 and Fig. 4). This suggests that availability of oxygen is an important determinant in killing by H_2O_2 and the sensitivity caused by defective NER is nullified to a large extent though not completely when oxygen availability is low. It is possible that low oxygen availability induces a general stress response which increases the tolerance to H_2O_2 killing. Alternatively, slower growth of cells in these conditions may allow a larger time window for other repair processes to act before the replication of DNA and subsequent cell division. Replication of damaged DNA is known to cause lethality because of the formation of breakages and collapse of replication forks [13]. It was reported that mode 1 killing by H_2O_2 (2.5mM for 15 min) in a wild type background was similar in both anoxic and aerated conditions and our data obtained with similar concentration of H_2O_2 and 60 min challenge is consistent with their findings [14]. However, at 90 min exposure non-aerated condition provided significant protection. It was also reported that growing cells in anoxic conditions before H_2O_2 challenge made *E. coli* cells sensitive to mode 1 killing by H_2O_2 [6] while we observed that low oxygen provided protection from mode 1 killing. The apparent contradiction in the results can be explained by the fact that in our study the cells were subjected to low oxygen conditions during the H_2O_2 challenge while in the previous study the cells were initially grown in low oxygen but experienced high oxygen conditions during the H_2O_2 challenge.

In our study, we have exposed the cells to H_2O_2 for longer time periods thus ensuring high levels of damage to DNA. It is possible that in this case the BER which is considered as the primary defence against oxidative damage to DNA was overwhelmed and hence NER became important for survival. This is supported by our result that *uvrA* cells showed moderately higher sensitivity than wild type to 2mM H_2O_2 but showed supersensitivity when exposed to higher concentration and for longer time period (Fig.1 and 2).

4. CONCLUSION

Overall these results strongly suggest that, nucleotide excision repair is involved in the repair of the oxidative stress-induced DNA damage. Nutrient deprived and low aeration growth conditions provide some protection against oxidative stress in *E. coli* but under these conditions too NER is important for survival. Previously, taking an in vitro approach and using purified enzymes it was proven that, (A)BC excinuclease removes two major products of oxidative damage, thymine glycol and the baseless sugar (AP site) from DNA [15]. This study however, lacked the in vivo data showing the contribution of this process to the survival of H_2O_2 induced killing. Our study fills this gap and provides the evidence that NER is indeed important for the survival of cells against killing by H_2O_2. Future studies monitoring changes in gene expression in response to oxidative stress in cells growing in starvation and anoxic conditions may shed light on underlying molecular mechanisms involved in providing cross-protection.

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