**katE** Complementation on **katG** Background has Negative or No Effect on the Ability to Protect Against UV-A-Mediated Killing in *Escherichia coli*

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In a previous study, **katE** complementation of an *E. coli** **katG** mutant suggested that an overexpression of **katE** was the cause for low survival after UV-A treatment. In this study, when JW strains lacked either **katE** or **katG** were complemented with **katE**, this overexpression was not observed. Rather, there were no differences between the two complemented JW strains after UV-A treatment. A possible theory on these phenomena suggests interference with other essential antioxidant genes or antioxidant inducer genes, such as **ahpC**, **oxyR** and **rpoS**, that need to be functional for **katE** and **katG** to be properly transcribed.

Within *E. coli*, two types of hydroperoxidases are produced to convert the toxic ROS into water and oxygen. These are known as HP1 and HPPII, which are encoded by genes **katG** and **katE** respectively (7). The levels of the two catalases respond to different stimuli because of the different shapes between HP1 and HPPII (4) and inducers for initiating protein synthesis (7). HP1 synthesis is induced by H$_2$O$_2$ and HPPII is induced during the transition from growth to stationary phase or just during growth phase with the presence of tricarboxylic acid cycle intermediates (7). In a previous study by Cheng et al., an attempt to complement **katE** and increase cell survival rate was tried. The investigation found that **katE**-complemented mutant had the lowest survival rate compared to other strains, which suggested either a fault in the complementation method or an alternative explanation (3). In this experiment, we will assess whether the expression of background in *E. coli* is important in expressing the heightened sensitivity to UV-A as previously discussed (3).

**MATERIALS AND METHODS**

**Bacterial strains and plasmid.** *E. coli* strains used are listed in Table 1. The plasmid pAMkatE72 used originates from Dr. Loewen (University of Manitoba, Department of Microbiology) and was used as a source of the **katE** gene. All the other strains were provided by Dr. Ramey (UBC, Department of Microbiology & Immunology). Wildtype cultures were grown in LB medium (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl for LB broth and an addition of 15 g/L yeast extract). 

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
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<tr>
<td>X$^{50}$</td>
<td>F-, araC14, leuB6(Am), secA206(aziR), fhuA23, lacY1, proC85, tiss-67, purE42, gbuV44(AS), gaiK2(Oc), LAM-, trpE38, xfaA15, his-208, rfbC1, mgl-51, argG77, rpsL109(strR), glpR201, xylA5, met-1, ilvA681, thi-1, metA160</td>
<td>University of British Columbia, Department of Microbiology &amp; Immunology, MICB 421 laboratory stock</td>
</tr>
<tr>
<td>BW25113</td>
<td>F-, Δ araD-araB)567, AlacZ4787(::mB-3), Δ (rhaD-rhaB)568, hsdR514</td>
<td>University of British Columbia, Department of Microbiology &amp; Immunology, MICB 421 laboratory stock</td>
</tr>
<tr>
<td>JW1721-1</td>
<td>F-, Δ araD-araB)567, AlacZ4787(::mB-3), Δ (rhaD-rhaB)568, hsdR514</td>
<td>University of British Columbia, Department of Microbiology &amp; Immunology, MICB 421 laboratory stock</td>
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<tr>
<td>JW3914-1</td>
<td>F-, Δ araD-araB)567, AlacZ4787(::mB-3), Δ (rhaD-rhaB)568, ΔkatG729::kan, hsdR514</td>
<td>University of British Columbia, Department of Microbiology &amp; Immunology, MICB 421 laboratory stock</td>
</tr>
<tr>
<td>CCHL08W-01</td>
<td>UM2 host with pAMkatE72 (3)</td>
<td>University of British Columbia, Department of Microbiology &amp; Immunology, MICB 421 laboratory stock</td>
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</table>
The transformed strains were grown in the same LB media with ampicillin (100 μg/mL) and kanamycin (100 μg/mL).

**DNA extraction of pAMkatE72.** Plasmid pAMkatE72 was isolated from CHL08W-01 previously described (3) using a modification of the phenol extraction and ethanol precipitation technique (1). 250 μL TE-saturated phenol chloroform (phenol (25): chloroform (24): isooamyl alcohol (1)) was mixed with 250 μL of strain CHL08W-01. DNA was extracted by vortex and centrifugation at 12,000 rpm for 5 minutes at room temperature. The top aqueous layer was isolated and kept. This process was repeated until 250 μL was reached. Then, 25 μL of 3M Na-acetate, 500 μL 100% ethanol was mixed and kept at -20 °C overnight for DNA precipitation. The sample was then centrifuged at 12,000 rpm for 15 minutes at 4 °C and resuspended to 50 μL with 3M NaCL after washing with 70% ethanol. DNA purity and concentration were determined by absorbance readings at 260 nm and 280 nm. The DNA was stored at -20 °C until needed.

**Transformation with pAMkatE72.** All E. coli strains except the CHL08W-01 strain listed in Table 1 were transformed using the following electroporation techniques modification of the protocol specified by the manufacture (2). Electrocompetent cells were prepared by inoculating 125 mL of LB broth with 10 mL of all strains for transformation. The cells were grown at 37 °C, shaken at 300 rpm, to OD₆₀₀ values between 0.5-0.7. Cells were then harvested via centrifugation at 4000 x g for 15 minutes at 4 °C, and resuspended in 0.5 mL of ice cold 10 % glycerol. For every 50 μL of cell suspension, 2 uL of DNA was mixed and pulsed at 2.5 KV. These cells were then resuspended in 1 mL of SOC medium (20 g/L tryptone, 5 g/L yeast extract, 0.5 g NaCl, 2.5 mM KCl, 20 mM MgCl₂). The cultures were incubated at 37 °C for 1 hour at 225 rpm, and then plated on LB agar medium with ampicillin (100 μg/mL) for transformant selection.

**UV-A treatment.** Three replicate samples of 500 μL fresh overnight transformants were each suspended in 1 mL M9 minimal nutrient broth (64 mg/mL Na₂HPO₄, 7H₂O, 15 mg/mL KH₂PO₄, 2.5 mg/mL NaCl, 5 mg/mL NH₄Cl, 0.001 mol/mL MgSO₄, 0.001 mol/mL CaCl₂, 0.02% glucose) in small petri dishes, and then plated on LB agar medium with ampicillin (100 μg/mL) at 20 °C until needed.

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the vicinity of katE/G could have become dysfunctional due to a mutation (7). oxyR and rpoS genes are essential in inducing the antioxidant mechanism (katE/G). Also, the function of ahpC gene, which is another major antioxidant gene, may have been damaged more in one JW strain than the other with complementation (7) due to differences in kat genes in each of the JW strains. The variations between replicates may be due to the insensitivity and imprecision of the “disc-floating” catalase assay method since the results for each set were similar but not precise. The direction or the path of the floating disc was difficult to control, which made the time measurements difficult as well.

FUTURE EXPERIMENTS

Future experiments could contribute to the findings of this experiment by testing the complemented and uncomplemented JW1721-1 strain with and without UV-A treatment to see if it is unaffected by UV-A. For more precise measurements of the catalase activity, other catalase assays that allow more control should be used at higher concentrations so that the dilution does not affect the comparability between enzyme activities of the lower concentrations. Additionally, increase in replicates will improve the credibility of the enzyme activity trends. As the results in this lab suggests that other antioxidant genes may be in play, future studies should look into these genes in detail and include them into the comparison analysis.

ACKNOWLEDGEMENTS

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REFERENCES