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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 HPII, 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 HPII (4) and inducers for initiating protein synthesis (7). HPI synthesis is induced by H_20_2 and HPII 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

TABLE 1. *E.coli* strains used

Strain	Genotype	Source
X^{760}	F-, *araC14*, *leuB6*(Am), *secA206*(aziR), *fhuA23*, *lacY1*, *proC83*, *tsx-67*, *purE42*, *glnV44*(AS), *galK2*(Oc), *LAM-*, *trpE38*, *xthA15*, *his-208*, *rfbC1*, *mgl-51*, *argG77*, *rpsL109*(strR), *glpR201*, *xylA5*, *mtl-1*, *ilvA681*, *thi-1*, *metA160*	University of British Columbia, Department of Microbiology & Immunology, MICB 421 laboratory stock
BW25113	F-, *Δ(araD-araB)567*, *ΔlacZ4787*(::rrnB-3), *&lambda⁻*,*rph-1*, *Δ(rhaD-rhaB)568*, *hsdR514*	University of British Columbia, Department of Microbiology & Immunology, MICB 421 laboratory stock
JW1721-1	F-, *Δ(araD-araB)567*, *ΔlacZ4787*(::rrnB-3), *&lambda⁻*,*ΔkatE731::kan*, *rph-1*, *Δ(rhaD-rhaB)568*, *hsdR514*	University of British Columbia, Department of Microbiology & Immunology, MICB 421 laboratory stock
JW3914-1	F-, *Δ(araD-araB)567*, *ΔlacZ4787*(::rrnB-3), *&lambda⁻*, *rph-1*, *Δ(rhaD-rhaB)568*, *ΔkatG729::kan*, *hsdR514*	University of British Columbia, Department of Microbiology & Immunology, MICB 421 laboratory stock
CCHL08W-01	UM2 host with pAMkatE72 (3)	University of British Columbia, Department of Microbiology & Immunology, MICB 421 laboratory stock

agar for LB agar, at pH 7.0). The transformed strains were grown in the same LB media with ampicillin (100 ug/mL) and kanamycin (100 ug/mL).

DNA extraction of pAMkatE72. Plasimd pAMkatE72 was isolated from CCHL08W-01 previously described (3) using a modification of the phenol extraction and ethanol precipitation technique (1). 250 uL TE- saturated phenol chloroform (phenol (25): chloroform (24): isoamyl alcohol (1)) was mixed with 250 uL of strain CCHL08W-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 uL was reached. Then, 25 uL of 3M Na-acetate, 500 uL 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 uL with dH_2O 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 CCHL08W-01strain 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 OD_{600} 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 uL 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 $MgCl_2$). The cultures were incubated at 37 ˚C for 1 hour at 225 rpm, and then plated on LB agar medium with ampicillin (100 ug/mL) for transformant selection.

UV-A treatment. Three replicate samples of 500 uL fresh overnight transformants were each suspended in 1 mL M9 minimal nutrient broth (64 mg/mL Na_2HPO_4 $7H_2O$, 15 mg/mL KH_2PO_4, 2.5 mg/mL NaCl, 5 mg/mL NH_4Cl, 0.001 mol/mL $MgSO_4$, 0.001 mol/mL $CaCl_2$, 0.02% glucose) in small petri dishes, and then simultaneously exposed to UV-A radiation for 90 minutes after concentration normalization via OD_{600} readings and dilutions to close to 4.00 OD_{600}. Immediately after UV-A treatment, catalase activity was measured by measuring the time for Whatman 540 disks 7 mm in diameter soaked in culture and dropped in 100 mL 1% H_2O_2 solution to rise 9.5 cm.

RESULTS

JW strains show different degree of reactions to UV-A treatment. *katE* complemented JW1721-1 and JW3914-1 strains were subjected under catalase activity before and after UV-A to assess the effect of complementation on ROS responses of the cultures. The catalase activities of the two JW strains are significantly different from those of the parent BW25113 strain and X^{760} as shown in Figure 1. BW25113 and JW 3914-1 showed similar enzyme activities to one another as well as X^{760} to JW1721-1 before UV-A exposure according to the column graph in Figure 2. All *katE* complemented strains showed increase catalase activity after UV-A exposure. However, unlike the two control strains, BW25113 and X^{760}, the JW strains did not result in enzyme activity patterns prior to UV-A treatment. These patterns in the increase of catalase activity also mimic each other as shown in Figure 2.

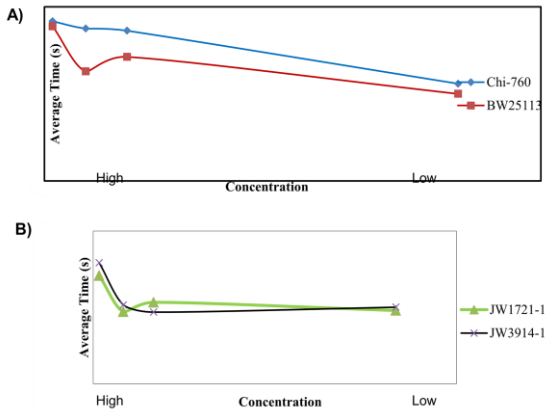

FIG 1. A. Catalase Activity Trend for transformed Chi-760 and BW25113 post UV-A treatment; B. Catalase Activity Trend for Transformed JW1721-1 and JW3914-1 post UV-A treatment.

DISCUSSION

From Figure 1A and 1B, it was determined that the lower concentrations were too diluted to show clear differences average time for the Whatman disk to rise. Also, the values at 0.5 dilution showed the most consistent and significantly different results. Therefore, the data shown in Figure 2 is the comparison of enzyme activities at 0.5 dilution only.

Contrary to the results observed in Cheng et al.'s study (3), the complemented *E. coli* strain lacking *katG* (JW3914-1) did not show over expression of catalase compared to the other strains. More precisely, the trends suggest that with complementation the lack of *katG* in the background decreases catalase activity compared to its full potential after UV-A treatment.

Interestingly, the catalase activities of transformants prior to UV-A exposure showed JW3914-1 to have the highest enzyme activity rate while the rest act similarly to each other as shown in Figure 2. Furthermore, this trend of strain JW3914-1 seems to disappear post UV-A exposure according to Figure 2. This result seems to suggest that it is *katE* that has a regulatory effect on *katG* rather than the other way around suggested by Cheng et al. (2) or that the complementation prevents the catalase stimulation by UV-A in the strain JW 3914-1.

A possible explanation for these unpredicted results could be due to the side effects of *katE* complementation. For example, *oxyR* and *rpoS* genes in

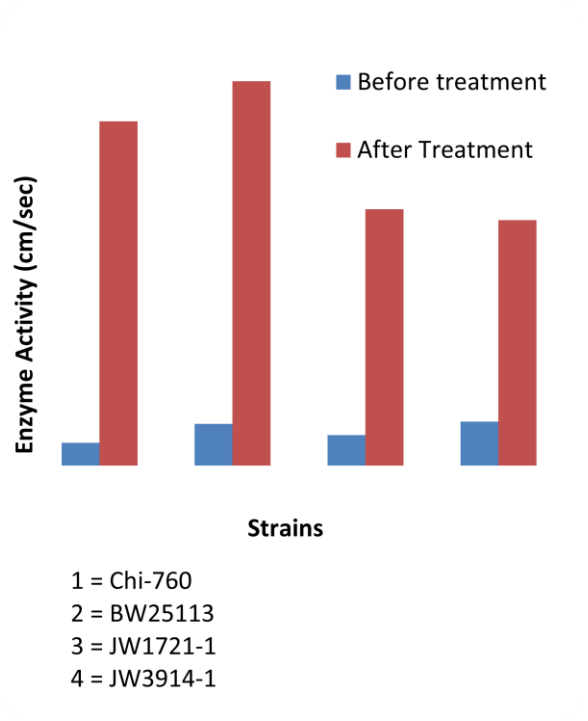

FIG 2. Enzyme activity rate for transformants before and after UV-A treatment at 0.5 dilution.

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.

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