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Transcription of *katG* is Enhanced in *Escherichia coli* exposed to UV-A and might Enhance Cell Survival.

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The solar water disinfection (SODIS) method relies on UV-A irradiation from natural sunlight for bactericidal treatment of drinking water. This study investigated the indirect effects of UV-A irradiation on *Escherichia coli* growth, death and gene expression. It has been previously shown that reactive oxygen species (ROS) induced by UV-A irradiation affect cell death rates. Three *E.coli* K12 strains – χ^{760}, UM2, JW3914 – were exposed to UV-A to investigate a potential correlation between UV-A-induced catalase expression and cell mortality. Turbidity and colony forming unit assays were performed to investigate cell growth and cell death respectively. As expected, an *E.coli* strain with *katG/katE* double knock-out showed a greater increase of death rate compared to the wild-type *E.coli* exposed to UV-A irradiation. However, differential catalase genotypes did not affect growth rate in our experimental model. A reporter system using a plasmid construct containing the *katG* promoter linked to detectable *luxCDABE* genes of *Photorhabdus luminescens* was used to examine the induction of *katG* expression in *E.coli* RFM443 by UV-A irradiation. *katG* expression was significantly up-regulated when *E.coli* incubated in water was exposed to UV-A irradiation; however, this increase was abrogated within Luria-Bertani media.

UV-A exposure is known to generate reactive oxygen species (ROS) such as superoxide, hydroxyl radicals and hydrogen via reactions with biological molecules (3). Excessive ROS are harmful to bacterial cells as the highly reactive oxygen molecules readily react and interfere with molecular processes within the cell in an uncontrolled manner (11). Solar water disinfection (SODIS) is a method used to disinfect drinking water by using natural sunlight to kill bacterial and viral pathogens **(**8**)**. Ultraviolet radiation from the sun is responsible for initiating the formation of ROS that in turn establish a bactericidal environment. Such affordable sterilization of drinking water makes this process ideal for developing countries **(**4).

E. coli is known to possess two genes, *katG* and *katE*, that produce catalase protein products (HPI and HPII respectively) that can protect the cell from the harmful effects of ROS (5,9). *E. coli katG* is part of the OxyR regulon that is induced by the presence of H_2O_2 (2). H_2O_2 oxidizes the OxyR protein inducing the transcription of *katG* and other components of the OxyR regulon (12). Previous experiments have shown that *E. coli* lacking *katE* are more susceptible to death mediated by UV-A exposure than are wild-type cells (1). *katE* is not a component of the OxyR regulon and its expression is unaffected by the presence of H_2O_2 (14). Double *katE* and *katG* knock-out strains have decreased survival rates relative to a wild-type parental strain under SODIS conditions, further suggesting the importance of the catalase genes in cell survival within a ROS rich environment (13). Together, this supports that gene product of *katG*, catalase HPI, is involved in a survival response to ROS.

The purpose of our experiment was to see if *katG* expression was up-regulated in *E.coli* in response to oxidative stress induced by UV-A irradiation. Further, we sought to explore whether this increase in *katG* expression would provide additional cellular protection against ROS and lead to increased survival under UV-A conditions. We found that *katG* expression was up-regulated and our data suggests that this likely has protective effects.

MATERIALS AND METHODS

Bacteria strains and growth conditions. *E. coli* K12 strains (Table 1) were obtained from the MICB421 bacterial strain collection (Department of Microbiology and Immunology, University of British Columbia). *E. coli* RFM443 containing pKatGLuxP reporter construct was obtained from The Hebrew University of Jerusalem. χ^{760}, UM2, and JW3914 were incubated and grown at 37°C on a shaking platform and RFM443 cultures were grown at 37 °C as previously indicated (2). All strains were grown in Luria-Bertani (LB) medium (1% w/v tryptone, 0.5% yeast extract, and 0.5% w/v NaCl, pH 7).

UV-A irradiation and growth assay. χ^{760}, UM2, and JW3914 strains were grown overnight in LB medium and transferred to fresh LB media prior to experiment to ensure cells were in exponential phase during the trial. A 1 ml sample from each culture was then placed into a petri dish containing 9 ml of fresh LB media. UV-A irradiation was performed at 37°C under UV-A light bulbs. Controls were incubated in darkness with no exposure to UV-A irradiation. Samples of each strain, incubated with or without UV-A exposure, were periodically measured at OD_{460} (Spectronic 20D+) for turbidity. Readings were also performed at the same temperature.

UV-A irradiation and death assay. χ^{760}, UM2, and JW3914 grown overnight in LB medium and re-inoculated in fresh

TABLE 1. *Escherichia coli* K12 strains used.

Strain	Genotype	Reference
χ^{760}	Wild-type for both *katE* and *katG;* parental strain for UM2	UBC MICB421 Laboratory
UM2	*katE2*; *katG15*	UBC MICB421 Laboratory
JW3914	*katG729::kan*	UBC MICB421 Laboratory
RFM443	Wild-type with pKatGLuxP	The Hebrew University of Jerusalem; Belkin Laboratory

LB media prior to experiment to ensure cells were in exponential phase during the trial. Cultures were then diluted to establish a cellular concentration of 1.5 x 10^4 cells per petri dish when 1 ml of culture was added to 19 ml of H_2O. UV-A irradiation was performed at 37°C under UV-A light bulbs. Controls were incubated in complete darkness with no exposure to UV-A irradiation. Samples were plated onto LB at various time points and death was assessed by plate counts.

RFM443 reporter assay monitoring *katG* expression. *E.coli* RFM443 was grown overnight in LB medium. The overnight culture was serially diluted and transferred to fresh LB media or H_2O in a clear plastic petri dish, resulting in final concentrations of 1/4, 1/40, and 1/400 of that of the overnight culture. Duplicate dishes were exposed to either UV-A irradiation, H_2O_2 at 20 mg/l, or darkness. Luminescence was measured by TD-20/20 luminometer. An OD_{460} reading was taken at the start and end of the time trial by a spectrometer (Spectronic 20D+).

RESULTS

Comparison of growth under UV-A exposure. UV-A exposure had no significant effect on the growth of χ^{760}, JW391 and UM2 in LB medium as measured by turbidity. OD_{460} readings at all time points were consistent across UV-A exposed and control samples of each *E. coli* strain. Fig. 1 highlights that all three strains, regardless of the presence of *katG* and *katE*, grew under UV-A exposure at similar rates from an OD_{460} of approximately 0.2 to a common final reading of approximately 0.9. These results are contrary to our expectations that that UV-A exposure would have an inhibitory effect on growth, particularly in strains not equipped with the *katG* and *katE* catalase genes.

Cell survival under UV-A exposure. Measurements of cell death proved to coincide more closely with our expectation that harboring *katG* would confer a survival advantage under UV-A exposure. χ^{760}, JW3914 and UM2 samples exposed to UV-A had increased death rates compared to control samples. Fig. 2A, 2B and 2C depict the decreasing CFU/ml observed in each sample. UM2 and χ^{760} exposed to UV-A had slightly steeper death curves indicating a more rapid death rate. Although the plate counts were highly variable for the JW3914 strain and could not be fit with an appropriate trend line, the trend is clearly downward. The JW3914

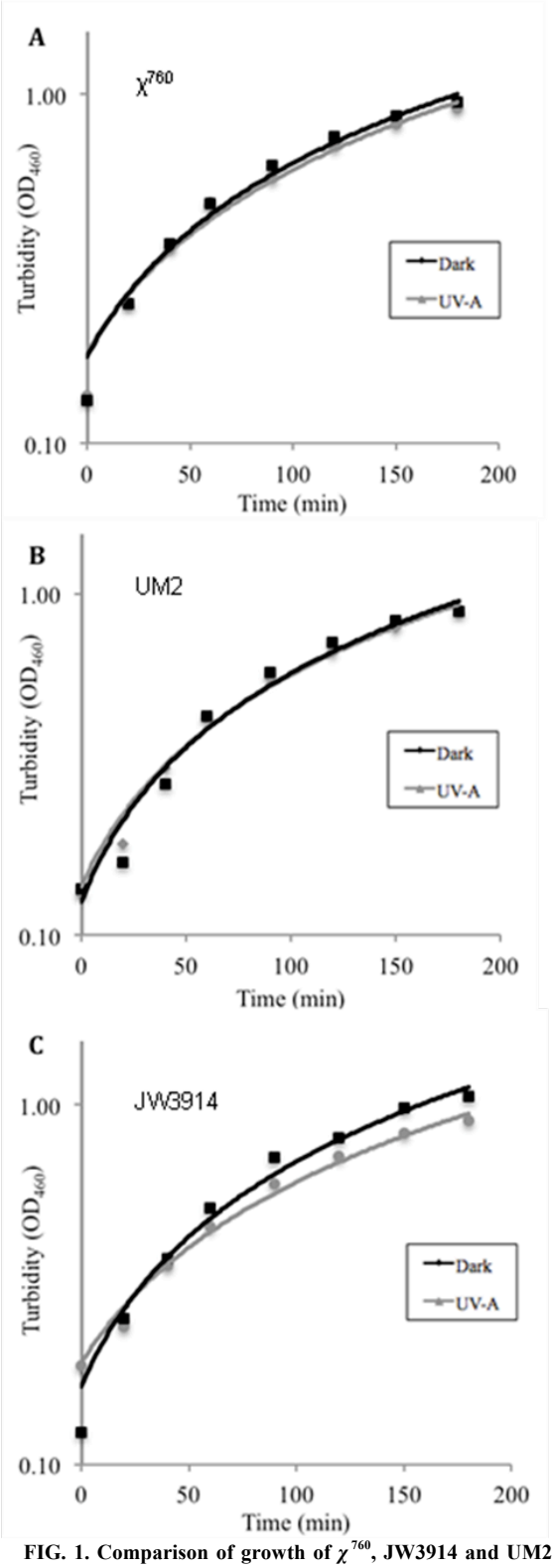

FIG. 1. Comparison of growth of χ^{760}, JW3914 and UM2 *E.coli* strains under UV-A exposure (experimental) and in the absence of UV-A irradiation (control). . (A) χ^{760}, (B) UM2, and (C) JW3914.

UV-A-exposed sample appeared to have a dramatically increased death rate compared to the control, but unfortunately this data was extrapolated from highly variable plate count data that cannot be considered reliable. Due to this high level of variability the JW3914 strain was not considered in further analysis.

Each sample started with a different initial cell concentration. Therefore, for comparison the CFU/ml data was normalized to a percentage of the CFU/ml at T=0 (Fig. 3). As expected, the UM2 strain had a more rapid decrease in cell survival compared to χ^{760}. This is clearly indicated by the steeper slope of the trend line in Fig. 3. The most dramatic difference in cell death rates appeared to occur between 0 and 25 minutes of exposure to UV-A. A 90% decrease in cell survival of the UM2 strain was observed within 40 minutes of exposure, compared to a 80% decrease observed in the χ^{760} strain. After 30 minutes the UM2 strain death rate appears to slow and between 40 and 60 minutes the death rates are almost identical. This observation is likely due to limitation of sensitivity in this experiment; as the number of cells decreased, the margins of error became more significant. Based primarily on the data collected in the first twenty minutes of this experiment, we suspect that that the death rates are significantly different. Unfortunately, due to the sensitivity of this experiment and the futility of JW3914 replicates this trend can be neither supported nor refuted by our data. This data supports that *katG* does convey an advantage for survival under UV-A exposure in H_2O.

***KatG* expression assay.** No increase in *katG* expression was observed in cells growing in LB and exposed to UV-A. The measured luminescence (RLU) was lower than in the dark control at all time-points. Appropriate activation of the reporter system was validated by cells exposed to H_2O_2 (Fig. 4A). Luminescence in these samples quickly increased and remained at high levels throughout the experiment. Lower initial levels of detected luminescence produced by cells in H_2O_2, compared to the dark control and UV-A exposed samples, was attributed to a lower starting cell concentration due to significant cell death indicated by OD_{460} readings. An OD_{460} reading taken at the end of the time trial indicated that the cell culture exposed to H_2O_2 was 60% as turbid as its initial reading. This indicated that cells could up-regulate *katG* under oxidative stress caused by reactive oxygen species such as H_2O_2. Contrary to expectations no change in *katG* expression was observed in cells exposed to UV-A when the cells were in LB medium. This result was explained by repeating the experiment in H_2O instead of LB media (Fig. 4B).

When cells were tested in water the positive control, cells exposed to H_2O_2 reacted the same way as in the previous LB replicate by increasing *katG* expression. The trend in the dark control was also as before. The experimental sample exposed to UV-A experienced increased luminescence indicating an up-regulation of *katG* by the cells as expected. The UV-A exposed cells reached a peak of luminescence at 40 minutes into exposure and a subsequent decrease. This result suggests that UV-A did form potentially damaging ROS and that the cells reacted to this stress by up-regulating *katG* expression.

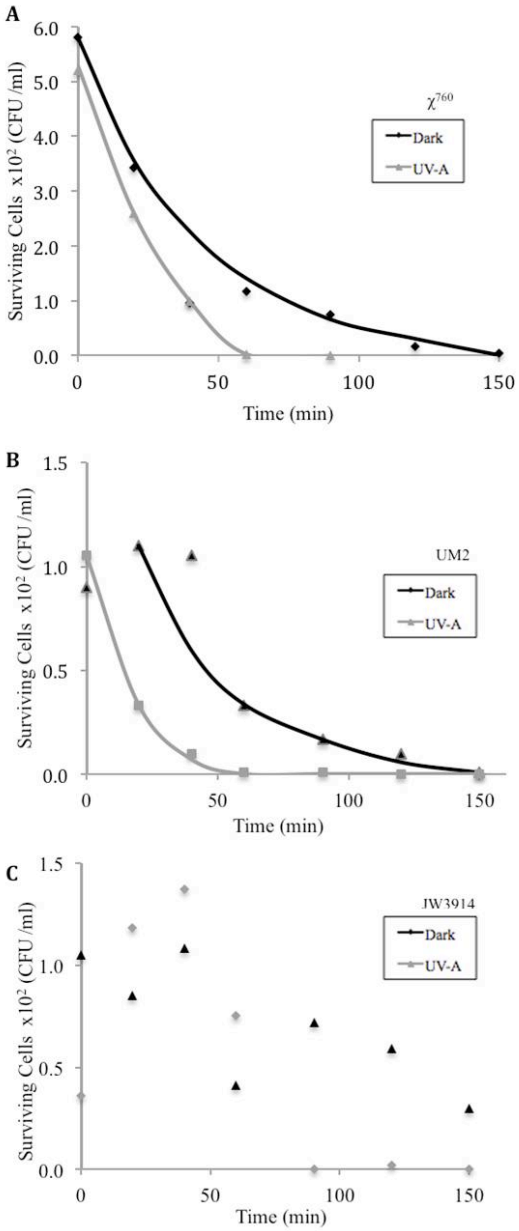

FIG. 2. Comparison of *E.coli* strain χ^{760}, UM2, and JW3914 survival under UV-A exposure. The curve of the trend lines indicate the rate of death. UV-A exposure was experimental condition and dark was used as a negative control. (A) χ^{760}, (B) UM2, and (C) JW3914.

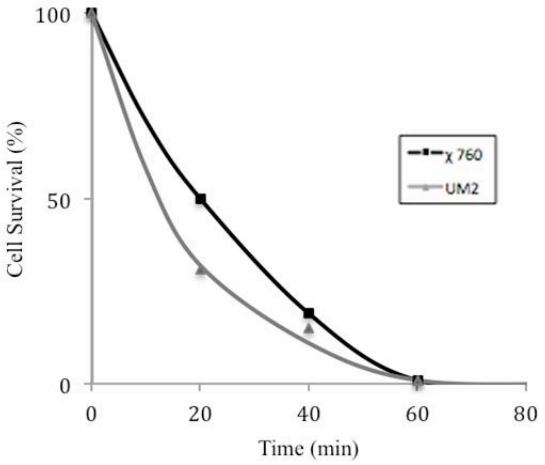

FIG. 3. Comparison of χ 760 and UM2 cell survival under UV-A exposure. Cell survival was normalized from CFU/ml data to a percent of CFU/ml observed at T=0.

DISCUSSION

The aim of this experiment was to assess whether *katG* contributes to increased survivability of *E.coli* under UV-A irradiation conditions. The basis for this experimental question was the widespread use of the SODIS water purification system that depends on UV-A induced ROS mediated killing of bacteria in H_2O. We were interested in how gene expression may increase or decrease bacteria's susceptibility to this method of water purification.

The first part of this experiment focused on exploring whether *katG* presence in the *E.coli* genome conveyed an advantage during exposure to UV-A irradiation. We hypothesized that excess ROS formation, induced by UV-A irradiation, would affect cellular growth and death rates. *E.coli* cells lacking *katG* were expected to display both decreased growth rates and increased death rates when compared to wild-type cells.

In general, all three strains tested, χ^{760}, JW391 and UM2 shared near-identical growth rates resulting in the high level of overlap observed in Fig. 1. Interestingly, no significant difference in growth rate was observed in χ^{760}, JW3914 or UM2 samples grown in LB medium while exposed to UV-A compared to control cultures incubated in the dark. This data suggests UV-A irradiation exerts no negative effects on the growth rate of *E. coli*. More specifically this suggests that the absence of *katG* and/or *katE* has no effect on the growth rate of *E.coli* under UV-A irradiation. However, our experimental model may have minimized the impact of ROS formed by UV-A on growing cells. The cellular concentration in the rich media was low respective to that of the molecular nutrients within the fresh inoculum. ROS induced by UV-A irradiation could have predominantly reacted with such compounds. Therefore the high nutrient concentration within the LB medium may have exerted an indirect protective effect from ROS, eliminating the need for induction of cellular defense mechanisms, such as the HPI catalase. Therefore, the effect of *katG* expression on growth rates under oxidative stress conditions remains inconclusive.

Contrary to the effect of UV-A on growth, UV-A was observed to have a negative effect on cell survival of χ^{760}, JW391 and UM2 incubated in H_2O under UV-A exposure compared to control samples incubated in H_2O the dark. This observation suggests that the UV-A irradiation accelerated the death rate. Cellular death could be attributed to either a direct result of ROS oxidizing effects, or the increased catabolic burden that is imposed upon the cell when damage is detected (12). Comparison between the UM2 and χ^{760} strains suggest that the expression of *katG* and/or *katE* resulted in protective effects against UV-A induced ROS. The UM2 strain experienced 90% cell death within forty minutes of UV-A exposure while the χ^{760} strain experienced 80% cell death within the same time interval. Based on the time interval and experimental design we consider this difference both considerable and reasonable. Unfortunately, the JW3914 strain data was compromised and not useful for further analysis which limits the conclusions we are able to make about the primary focus of our investigation, *katG*. We expect that if this experiment was repeated to yield more reliable results the JW3914 strain would experience an increased death rate compared to a wild-type control.

The second part of this experiment examined whether wild-type *E.coli* up-regulated *katG* expression in response to UV-A irradiation. Unexpectedly no induction of *katG* expression was observed in wild-type cells exposed to UV-A in comparison to the dark control in LB media. The legitimacy of our reporter system was validated by exposing cells to H_2O_2 and the observation of high levels of luminescence. This indicated that the cells were capable of up-regulating *katG* expression in response to oxidative stress yet did not do so in response to UV-A exposure. Similar to our experiment testing the effect of *katG* on growth rate, the low cellular concentration relative to the molecular components within the LB media may be the cause for lack of *katG* induction and expression. This expectation was supported by repeating the experiment incubating samples in H_2O.

The expression of *katG* when cells were incubated in H_2O followed trends previously observed *when E. coli* cells were introduced to sub-lethal oxidative stress (13). Cells that were exposed to UV-A irradiation had a lag time of approximately 20 minutes before increased luminescence was observed indicating an up-regulation in *katG* expression. One possible explanation for the

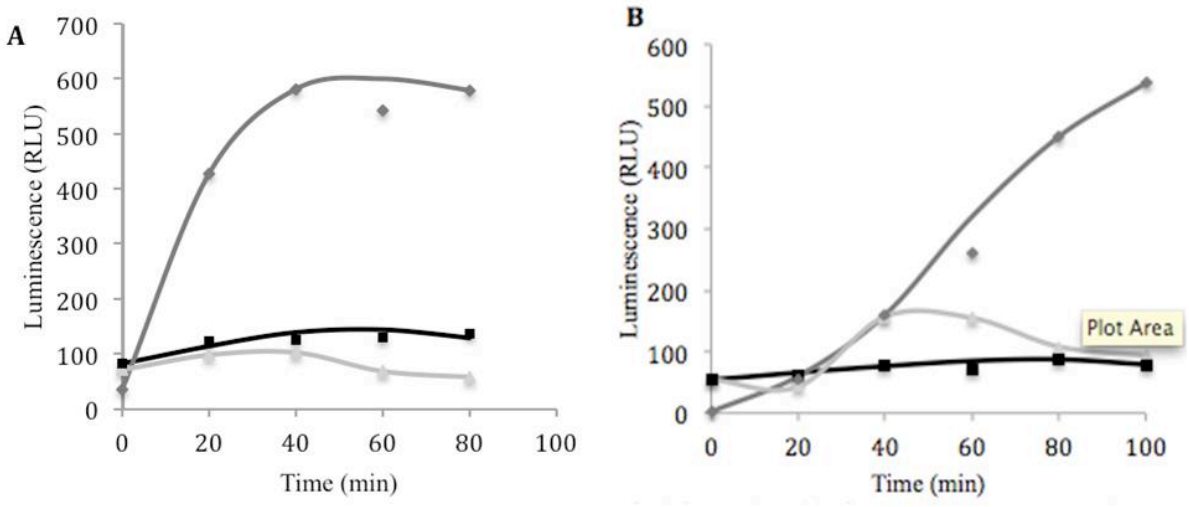

FIG. 4. Comparison of *katG* expression measured by luminescence of Lux reporter system in *E.coli*. Legend: Dark control (black), H_2O_2 (dark grey) and UV-A (light grey). (A) *E. coli* RFM443 containing pKatGLuxP construct incubated in LB media, challenged with H_2O_2 and UVA. (B) *E. coli* RFM443 containing pKatGLuxP construct inoculated in H_2O, challenged with H_2O_2 and UVA

katG expression lag time experienced under UV-A conditions is the time required for UV-A irradiation to produce ROS at a quantity large enough to stimulate an OxyR mediated response to oxidative stress. After the initial increase in *katG* transcription seen at 40 minutes, the elevated levels of *katG* transcription from 40 to 60 minutes plateaued and then decreased to levels comparable to those of cells not exposed to UV-A irradiation. This trend may be explained by the biological properties of the OxyR protein. Although only oxidized active OxyR can induce *katG* transcription, both the inactive and active form of OxyR have been shown to act as repressors for transcription of the *oxyR* gene (13). Although ROS may have initially activated *katG* transcription, the quantity of ROS produced by UV-A irradiation may have been insufficient to evade HPI activity and ensure constant OxyR oxidation and hence continued *katG* expression, as seen in the cells exposed to a high concentration of hydrogen peroxide.

SODIS depends on the production of ROS initiated by UV light to effectively eliminate viable microorganisms (11). Our results indicated that the presence of *katG* can influence the rate at which bacteria succumb to such conditions and that *katG* transcription was up-regulated in *E. coli* when the cell was placed in H_2O and exposed to UV-A light. We propose that there are other factors that may influence cells survivability under SODIS-like conditions, such as total cellular concentration and molecular components of the surrounding media, both of which potentially alter the ROS burden experienced by the individual cell. In order for SODIS procedures to optimize its disinfection ability, such factors should be investigated further.

FUTURE DIRECTIONS

This experiment focused on a single protective response in *E.coli*. However, *E.coli* up-regulates a large number of proteins in response to oxidative stress and many additional protection mechanisms could be investigated including cellular mechanisms of preventing ROS generation, quenching of chain propagation and repair of damage caused by ROS. For example, the OxyR protein regulates up to 30 different genes (13). In addition, the SoxR regulon has also been shown to control nine proteins involved in protection from ROS such as a manganese-containing superoxide dismutase, which destroys superoxide radicles, and endonuclease IV, which repairs ROS-induced DNA damage (7). By using the same principles indicated in this experiment to create an expression construct, the level of transcription of different repair or protective proteins could be monitored in different stressful cellular environments. These types of investigations could provide insight into which protective mechanisms are important under certain conditions and perhaps support or refute the importance of the role of *katG* in protection against ROS.

The goal of applying the concepts explored in this experiment to the SODIS system is to improve its efficiency by increasing our understanding of bacterial defense mechanisms that may inhibit UV mediated killing. We have determined that UV-A causes cell death via induction of excessive ROS and that this is countered by *katG* expression. This conclusion provides

a valuable link for future experiments. An important future experiment would be to utilize the experimental methodology outlined in this experiment and expand the variables considered. Previous research reported success in increasing temperature to 55°C, which is the estimated temperature that water in SODIS containers reach under equatorial sunlight, to eliminate *E.coli* contamination (6, 10). Our experiment could be elaborated on by exposing wild-type cells harbouring the pKatGLuxP reporter construct to various conditions that may be encountered during SODIS treatment, such as temperature, pH and organic compounds, and measuring cell death under UV-A exposure as well as *katG* expression. The death assay portion of this experiment will indicate whether the variable increases or decreases the killing power of UV-A while the expression assay will indicate whether the change in efficiency of UV-A irradiation is due to a change in the amount of ROS being produced.

Finally, our results have suggested that the non-microbial contents of the water play an important role in the initiation of *katG* transcription. By manipulating the concentration of certain components within the medium, insight may be offered into the protective effects of individual molecules. Such information would be useful in determining the amount of time required to eliminate water of viable microbes from different environmental sources.

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