The Role of Exogenous Sensitizers in the Photoinactivation of Pathogenic Bacteria

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With approximately 100 million Americans using the marine environment for recreation each year, one of the primary concerns in public health has become the risk that humans will encounter pathogenic bacteria when these water become contaminated. Contamination of these waters can occur in a number of ways, including stormwater runoff, industrial waste, and sewer systems. In order to best understand the possible health concerns, the life of the bacteria in environmental waters must be examined.

Environmental waters contain natural organic matter that can acts as what is known as a photosensitizer. In order to understand the fate of bacterial pathogens in environmental waters it is necessary to understand how these photosensitizers might affect the photoinactivation of bacteria. Photoinactivation within bacteria can occur in three ways, all of which damage the cell's DNA and its ability to replicate: 1) sunlight can direct hit the DNA, 2) sunlight can indirectly hit a sensitizer inside the cell wall, or 3) the sunlight can indirectly hit a sensitizer outside the cell wall. In the indirect cases, the DNA is damaged by the reactive oxygen species (ROS) that are generated by the photosensitizers.

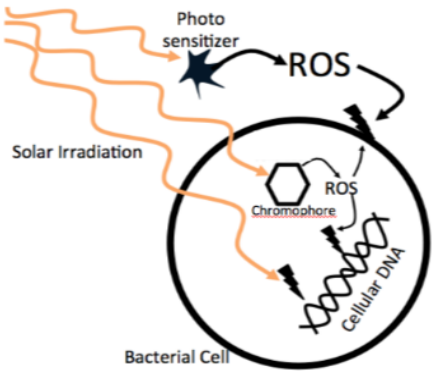

Figure 1. Three pathways for bacterial photoinactivation

It is important to note that whatever sunlight is being absorbed by the exogenous sensitizer is sunlight that is no longer reaching the cell itself. Therefore the presence of exogenous sensitizers can either aid the photoinactivation of the bacteria by generating ROS or hinder the photoinactivation of bacteria by blocking out sunlight.

Bacteria are classified as either gram-positive or gram-negative depending on the structure of their cell wall. The gram-negative bacteria cell wall contains an additional outer membrane that is made of lipopolysaccharide. By using gram stain as the defining characteristic in testing the photoinactivation of bacteria, Stanford University PhD student Peter Maraccini was able to conclude that the properties of the cell wall may play an important role in determining whether a bacteria is or is not susceptible to exogenous photoinactivation.

It was this conclusion that motivated further research on different factors that may affect the interaction between the exogenous photosensitizer and the bacteria cell wall. This research aims to examine how the charge of various exogenous sensitizers may affect the photoinactivation of gram-positive and gram-negative bacteria.

In order to do this, two types of bacteria were each tested in three different experimental solutions. The bacteria used were gram-positive *Enterococcus faecalis* and gram-negative *Escherichia coli K12*. The experimental solutions used were carbonate buffer saline (CBS), rose bengal (RB), and methylene blue (MB). CBS acts as a control with no photosensitizers present, RB is a synthetic source of anionic sensitizers, and MB is a synthetic source of cationic sensitizers.

For both *Enterococcus faecalis* and *Escherichia coli K12* chemostats were established to grow the bacteria with tryptic soy broth and hold the population growth rate at the stagnation phase. This yields a constant source from which to take the bacteria from while running experiments.

Once the chemostat is established, a sample is taken, centrifuged, washed, and re-suspended in CBS. This sample is now known

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as the bacterial inoculant for the experiment. The bacterial inoculant is then added to one of the three experimental solutions. The prepared solution is then exposed to solar spectrum light in the solar simulator. For the duration of the experiment, samples of the prepared solution are taken at pre-determined intervals. Then samples are then plated onto tryptic soy agar at different dilutions (1, 1/10, 1/100, and 1/1000) and placed in the 37°C room to incubated. After about 24 hours the number of colony forming units (CUFs) on each plate are counted and the photoinactivation rate for each bacteria in each solution is determined.

CBS	49.50mL 1mM CBS
	0.50mL bacterial inoculant
RB	49.4708mL 1mM CBS
	0.0292mL RB stock solution
	0.50mL bacterial inoculant
MB	49.4654 1mM CBS
	0.0346 1/10 MB stock solution
	0.50mL bacterial inoculant

Figure 2. Experimental solution recipes

For both the gram-positive and gram-negative bacteria, the cationic MB solution resulted in a faster photoinactivation than the anionic RB solution. But what is more interesting is how these results compare to that of CBS. In the gram-positive bacteria it was noticed that both the RB and MB solutions resulted in faster photoinactivation than the CBS solution, suggesting that the their presence is aiding the photoinactivation of the bacteria. However, in the gram-negative bacteria it was noticed that only the MB solution resulted in faster photoinactivation than CBS while the RB solution resulted in a slower photoinactivation than CBS. What this suggests is that the presence of the cationic MB solution aided the photoinactivation while the anionic RB solution hindered the photoinactivation. Similar results were noticed when the same bacteria in the same experiment solutions were exposed to light under a 320nm cutoff filter. In correspondence with other published works, these results suggest that cationic photosensitizers may have a broader application in the photoinactivation of bacterial cells than anionic photosensitizers because of their ability to affect both gram-positive and gram-negative bacteria.

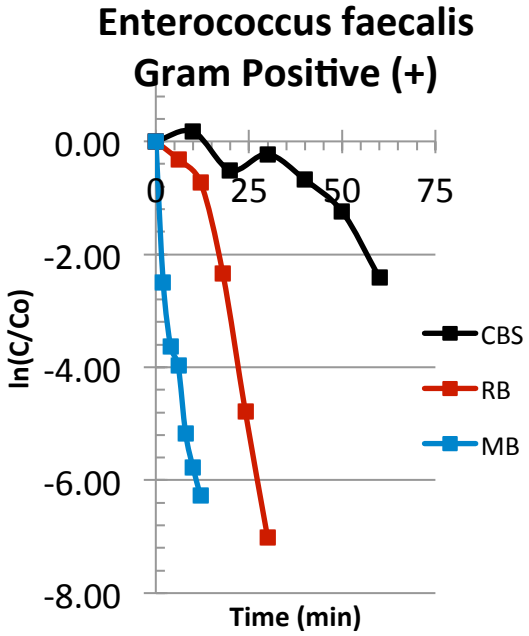

Figure 3. Photoinactivation of Enterococcus faecalis under full spectrum irradiation.

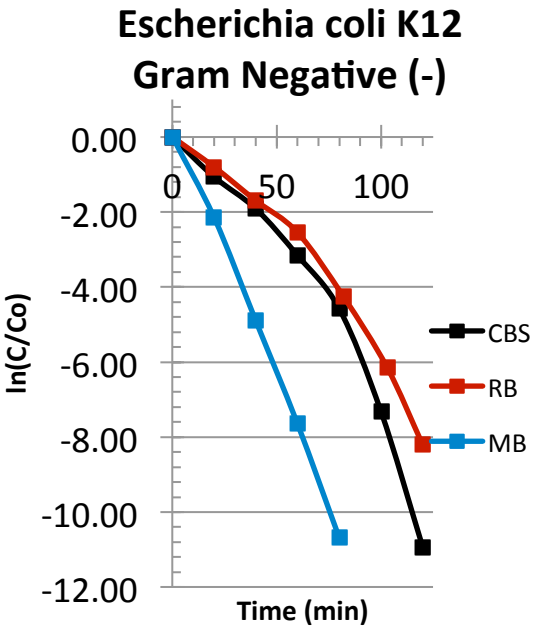

Figure 4. Photoinactivation of E. coli K12 under full spectrum irradiation.

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