"A Comparison of the Sensitivities of *Serratia marcescens* and *Deinococcus radiodurans* to Ultraviolet Irradiation"

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ABSTRACT

A simple, engaging bacterial mutagenesis exercise is described. This activity is designed to compare the effects of UV irradiation on a commonly-occurring, mildly pathogenic, pigmented microorganism, *Serratia marcescens*, to that of an unrelated, non-pathogenic, pigmented microorganism, *Deinococcus radiodurans*. Students inoculate TSA plates with microorganism, irradiate with UV light for specified times, and subsequently score plates for numbers and phenotypes of microorganism. Results demonstrate a dose-dependent sensitivity of *Serratia marcescens* cultures to UV-irradiation, with lethality as the most commonly scored phenotype. By contrast, similarly treated *Deinococcus radiodurans* cultures are resistant to the effects of UV irradiation over the length of exposure tested. The results are discussed relative to possible differences in management of oxidative stress.

INTRODUCTION

In recent years, there has been an increased interest in control of microbial growth, especially in light of single and multiply drug-resistant "superbugs" (1). Most students are also aware of the side effects associated with exposure to ultraviolet (UV) radiation. As an extension of the typical student exercise that explores physical control of microbial growth by means of UV irradiation, I have developed a simple, engaging exercise for introductory biology students. The exercise compares the level of sensitivity displayed by *Serratia marcescens* to that displayed by *Deinococcus radiodurans* in response to exposure to UV irradiation.

Serratia is a common, mildly pathogenic, pigmented microorganism, a member of the Enterobacteriaceae family within the Gamma-proteobacteria group (2). Its sensitivity to UV-irradiation is well known.

Deinococcus is an unrelated, non-pathogenic, pigmented organism, a member of the Deinococcaceae family of the Deinococcus-Thermus group (2). Its resistance to UV and gamma-irradiation is well documented (3).

Results of the experiments described here demonstrate a dose-dependent sensitivity of *Serratia* to UV irradiation, with lethality as the most commonly scored phenotype, and, rarely, loss of pigment. By contrast, *Deinococcus* cultures are resistant to the levels of UV exposure employed in these experiments and no other phenotypes observed. Such differences likely reflect differences at the cellular level.

MATERIALS AND METHODS

Organisms and Media

The *Deinococcus* strain used is ATCC 13939, as purchased through Microbiologics (MBL 0210P). The *Serratia* strain used is ATCC 29632, as purchased through Wards Scientific (D1). Media was prepared with deionized water, using standard Difco™ Tryptic Soy Agar (TSA-Fisher Scientific reference 236950) for inoculation of plates; or, BBL™ Trypticase Soy Broth (TSB, Fisher Scientific reference 211768) for broth cultures and dilutions.

Preparation of Experimental Cultures

Five milliliters of TSB was inoculated with one , well isolated colony and incubated for 24-36 hours at 25-30°C (to encourage the expression of pigment formation in the *Serratia* as well as to optimize growth of *Deinococcus*). These stationary phase cultures were diluted to achieve the optimum amount for plating: for *Serratia*, the culture was diluted 10^5-fold; for *Deinococcus*, 10^3-fold . One hundred microliters of diluted culture was plated for each time-point.

Treatment and Growth

After inoculated plates had dried completely, they were exposed to short wavelength UV (254 nm), using a Model UVG-54 Mineralight Lamp (UVP, Inc.) for specified times (unexposed control, 10, 20, 30, 40, 60, 80 and 120 second exposure). The intensity of irradiation was reduced to approximately 0.06 Watts (Joules sec^{-1}) by placing the lamp one meter above the exposure surface. Following exposure to UV, the lids were placed back onto the plates and cultures incubated at 25-30°C for 24 hrs (*Serratia* cultures) or 48 hours (*Deinococcus* cultures), and then scored for numbers/phenotypes.

RESULTS

Figure 1A. Response of *Serratia marcescens* to increasing times of exposure to UV light

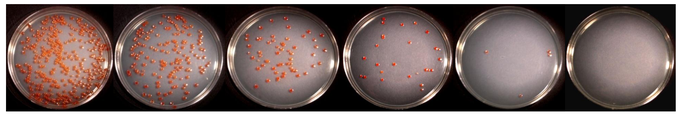
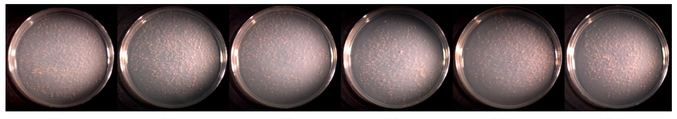


Figure 1B. Response of *Deinococcus radiodurans* to increasing times of exposure to UV light



Figures 1A, B. Microorganism plated and exposed to UV light for times (in seconds) indicated below images.

Organism	Number of Colony Forming Units (CFU)							
	Exposure to UV (seconds)							
	0	10	20	30	40	60	80	120
S. marcescens	259.6 ± 18.4	178.8 ±15.0	116.6 ± 8.0	81.6 ± 4.0	43.8 ± 2.7	11.6 ± 2.8	3.0 ± 0.9	0.0 ± 0.0
D. radiodurans	237.8 ±17.5	249.6 ±12.4	241.6 ±5.6	219.8 ±8.9	235 ±14.0	242 ±19.5	249.2 ±14.5	225.2 ±11.7

Table1. *Response of Organism to UV irradiation.* This data set is compiled from five replicates of one class and represent a typical profile. The number of CFU is expressed as mean ± standard error.

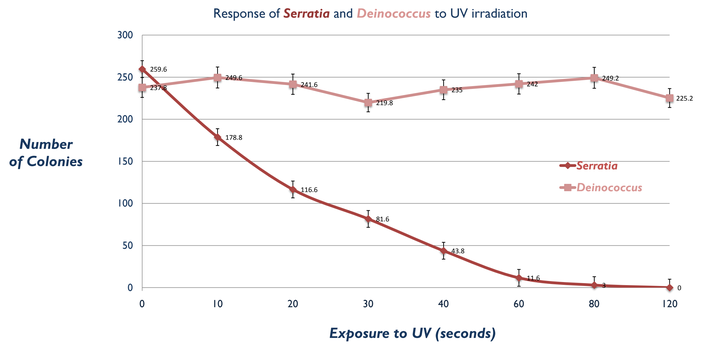


Figure 2. *Response of Serratia and Deinococcus to UV irradiation*. Data of five replicates is plotted, with mean values cited and standard error bars centered around each data point.

The results of a single set of plates inoculated with *Serratia* are compared to a single set of plates inoculated with *Deinococcus* in Figure 1. While the *Serratia* series (Figure 1A) clearly demonstrates a dose-dependent sensitivity to UV (scored as a decline in numbers of observable colony forming units), the *Deinococcus* series appears to be resistant (no statistically significant reduction in cfu) at the exposures tested. In addition to the most commonly observed phenotype (lethality) in *Serratia*, smaller, more slowly growing variants of *Serratia* have been scored (data not shown). To a far lesser extent (less than 0.0001%), non-pigmented variants are observed (data not shown). No phenotypic changes have been observed in any of the *Deinococcus* cultures.

Data from five replicates (five different student groups of the same lab section of the same date) is presented in Table 1 and graphed in Figure 2. Over the range of exposures to UV irradiation tested, *Serratia* is sensitive in a dose-dependent manner and *Deinococcus* is resistant to UV-mediated change. The results presented are typical and represent statistically significant differences between the two strains ($R^2 = 0.902$ for *Serratia* series; $R^2 = 0.041$ for the *Deinococcus* series).

DISCUSSION

This poster presents details of an exercise that is an extension of a standard laboratory experiment in the undergraduate laboratory relating to the physical control of microbes by means of UV irradiation (4). It explores a comparison between a commonly occurring, pigmented, mildly pathogenic organism (*Serratia marcescens*) with that of radiation resistant, non-pathogenic, pigmented organism (*Deinococcus radiodurans*). Recent studies support the idea that this resistance is due in large part to a reduction in radiation-induced protein oxidation and is likely mediated by a mechanism of protein protection offered by an accumulation of Mn(II) (5). The data presented here do not address the underlying mechanism of resistance (the phenotype scored is colony number/appearance and would miss specific but cryptic mutations). In spite of this limitation and variability in student performance, the data are consistent with the hypothesis that *Serratia marcescens* is sensitive to UV irradiation and *Deinococcus radiodurans* is resistant. I offer this exercise as a novel alternative to the more traditional exercises in bacterial mutagenesis.

REFERENCES

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