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Characterization of a Novel DNA Repair Phenotype in *Pseudomonas aeruginosa* Bacteriophage UNL-1

by

Julie J. Shaffer

A DISSERTATION

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GRADUATE COLLEGE
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Julie J. Shaffer, Ph.D.

University of Nebraska, 1999

Advisers: Eugene L. Martin and Kenneth W. Nickerson

UNL-1, a lytic bacteriophage of *Pseudomonas aeruginosa* isolated at the University of Nebraska, possesses a novel DNA repair phenotype. Bacteriophage DNA repair is quantified by Weigle reactivation, an experimental method in which UV induced bacterial host cells are infected with UV-C irradiated virus and plaque forming units enumerated. Previously, Weigle reactivation has only been observed in *P. aeruginosa* when host cells containing UV resistance plasmid R-2 and a functional *recA* gene are treated with UV-C radiation before bacteriophage infection. When UNL-1 is damaged with UV-C radiation to 1% infectivity, it is capable of over 10-fold reactivation when infecting *P. aeruginosa* host cells that have been previously exposed to UV-A radiation. However, the virus does not reactivate if *P. aeruginosa* is instead exposed to UV-C radiation. The reactivation is also not dependent upon a functional *rec A* gene or a UV^R plasmid, as shown previously, supporting the hypothesis that this is a new DNA repair phenotype. UNL-1 Weigle reactivation has also been shown to be synergistic to photoreactivation, so when incubating the pour plates in the light, a seventy-fold reactivation results. The gene or genes responsible have not yet been identified, but the

activity seems to be dependent upon host excision repair and at least partially upon the *rec 2* gene, which plays a role in *rec A* regulation. Although the exact nature of this repair is still unknown, this virus has been shown to reactivate if the host is first exposed to sunlight. It also shows some evidence of providing UV resistance to host cells when the bacteriophage is pseudolysogenic or in a semi-stable interaction with the cell. These interactions result in the production of brown pigment in 1-10% of the pseudolysogens. The phenotype changes in pseudolysogens containing UNL-1 could be important in understanding the survivability of some viruses in the environment and the interaction of bacteriophage with their host cells.

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Literature review

Almost 25 years after the discovery of plant viruses, F. W. Twort first published his discovery of a filterable agent that caused bacterial cultures to become "glassy and transparent" (Twort, 1915). In 1917 similar findings were published by Felix d'Herelle on a filterable agent that killed "dysentery bacillus" (see Duckworth, 1987). These discoveries began the study of bacterial viruses or bacteriophage, meaning bacteria eater.

Bacteriophage are divided into two categories based upon replication: lytic or lysogenic (Hayes, 1968). Lytic phage infect a bacterial cell, run the viral replication cycle, and are released by lysis of the cell. The model system for a lytic phage is *Escherichia coli* phage T4 (Campbell, 1996). Lysogenic phage or prophage, after infecting the cell, integrate their DNA into the host genome. Prophage DNA is then replicated along with the host DNA, and every daughter cell contains phage DNA. The prophage DNA can be activated to the lytic cycle, where the phage replicates and lyses the cell to release the new phage particles. This type of infection is exemplified by *E. coli* phage λ (Campbell, 1994).

Pseudolysogeny

A third type of cell-phage interaction, pseudolysogeny, has been noted but little studied. Pseudolysogeny was first reported in 1925 by McKinley as an alternative to lysogeny (see Baess, 1971). In pseudolysogeny the lytic bacteriophage genome remains

as an episome in the bacterial cell, neither replicating or entering the prophage state. Since the phage genome does not replicate along with the bacterium, only a small part of the population is infected at any one time. A portion of the infected bacteria will sporadically undergo phage induction, and the released phage infect the sensitive cells surrounding the lysed cell. In this way the infection is maintained in the bacterial population (Baess, 1971). Pseudolysogeny seems to arise due to sub-optimal growth conditions for the host cell (e.g. starvation) (Ripp and Miller, 1998).

Lysogenic conversion

Lysogeny and pseudolysogeny play important roles in host cell phenotype. Multiple examples of lysogenic conversion occur in different strains of bacteria (Table 1) (Ackermann and DuBow, 1987). Lysogenic conversion is a change in bacterial cell phenotype due to the presence of a prophage. In lysogenic conversion the bacterial host can be modified with regard to antibiotic resistance, antigen differences, mucoidy, and toxin production. All of these factors contribute to a bacterium's ability to withstand the immune system in the body, but not all of these phenotypic conversions are due to lysogeny. Some of the interactions are not very stable and may be due to pseudolysogeny. For instance, lytic phage are thought to influence the synthesis of toxin production in *Streptococci* sp. through pseudolysogenic interactions (Nida and Ferretti, 1982).

Table 1

Examples of lysogenic conversion

Bacterium	Phages	Conversion	Reference
Bordetella parapertussis	134	O antigen, toxin production	Lapaeva *et al.*, 1982
Escherichia coli	$o\beta_1$	Entertoxin production	Takeda and Murphy, 1978
Salmonella sp.	Numerous	O antigen	Smith and Parsell, 1974
Shigella flexneri	PE5	O antigen	Borek and Ryan, 1973
Yersinia pestis	P1	$Chloramphenicol^R$	Borek and Ryan, 1973
Myxococcus xanthus	Mx1	Abnormal motility and fruiting	Ruiz-Vazquez and Murillo, 1984
Pseudomonas aeruginosa	6 phages	Mucoid growth	Miller and Renta Rubero, 1984
Rhizobium trifolii	7, 7cr	O antigen	Borek and Ryan, 1973
Vibrio cholerae	Type II	O antigen, $polymyxin^S$	Milyutin *et al.*, 1980
Bacillus amyloliquefaciens	PK	Phage resistance	Jonasson *et al.*, 1969
B. cereus	wx23, wx26	Megacin A production	Ivánovics *et al.*, 1976
B. pumilus	PMJI, PMBI	Sporulation	Keggins *et al.*, 1978
B. subtilis	ρ11	Prototrophy	Dean *et al.*, 1976
B. thuringiensis	TP-13	Sporulation, crystal production	Perlak *et al.*, 1979
Clostridium botulinum	CEβ, DEβ	toxin production	Borlek and Ryan, 1973
Corynebacterium diphtheriae	β	Diphtherial toxin production	Borlek and Ryan, 1973
Mycobacterium smegmatis	D29	Lipase production	Borlek and Ryan, 1973
Staphylococcus aureus	ϕD	Staphylokinase producion	Borlek and Ryan, 1973
	LS1, LS2	Coagulase production	Duval-Iflah *et al.*, 1977
	42D	Enterotoxin production	Borlek and Ryan, 1973
Streptococcus pyogenes	ϕTg121	Erythrogenic toxin production	Borlek and Ryan, 1973

Adapted from Ackermann and DuBow, 1987.

Bacteriophage ecology

As seen in Table 1, phage are important in understanding bacterial infection and generating a treatment plan for certain human diseases. Bacteriophage are also important in controlling bacterial populations (Heldal and Bratbak, 1991; Proctor and Fuhrman, 1990; Suttle, 1994). It is estimated that 20% of marine heterotrophic bacteria are infected by phage, which lyse 10-20% of the bacterial population daily (Heldal and Bratbak, 1991; Suttle, 1994). Proctor and Fuhrman (1990) hypothesize that as high as 70% of all prokaryotes are infected by viruses in certain marine environments. Because heterotrophic bacteria are primarily responsible for the consumption of organic matter in marine waters (Azam *et al.*, 1983), this evidence suggests phage are important players in carbon and nitrogen cycling in the ocean.

Because phage likely play a key role in C and N cycling, it is important to understand the rates and mechanisms of decay of free phage or reduction of infective viruses in the water column. Suttle and Chen (1992) estimated the decay rates for three different phages in seawater. They found three main contributors to phage decay: i) adsorption by heat-labile particles, ii) consumption by flagellates, iii) solar ultraviolet (UV) radiation.

UV is divided into three main categories. UV-C or far UV is from 190-290 nm; UV-B or mid UV is from 290-320 nm; and UV-A or near UV is from 320-400 nm (Jagger, 1985). These ranges are not exact in that there is some gray area at the

intersections. Solar UV consists of UV-A and UV-B wavelengths. The UV-C gets filtered out by the ozone layer and is therefore of little consequence in the environment.

Decay rates of phage in the absence of UV were 0.009 to 0.028 h^{-1}, but with the addition of UV the decay rates were as high as 0.8 h^{-1} (Suttle and Chen, 1992). Viruses incubated in microcosms at the surface of the water can decay at a rate as high as 0.11 h^{-1} (Wommack *et al.*, 1996). Jeffrey *et al.* (1996) contributed to this area of study by measuring phage decay during calm seas versus seas with moderate wave action. In calm sea conditions UV damage was highest at the surface but reached depths of 10 m. In moderate sea conditions there was no apparent UV damage even at the surface. This supported an earlier model that suggested mixed-layer depth and the UV attenuation coefficient were important factors when calculating virus mortality in water columns (Murray and Jackson, 1993). An interesting development in this research area occurred when Noble and Fuhrman (1997) demonstrated that native viruses were more resistant to UV killing than nonnative viruses in the Santa Monica Bay. The maximal decay rate for nonnative viruses by solar radiation was $\frac{1}{3}$ to $\frac{2}{3}$ of the total decay rate and for native viruses $\frac{1}{4}$ to $\frac{1}{3}$ of the total decay rate.

Damaging effects of solar UV

Most of the damage to phage from solar radiation is from UV-B radiation (320-280 nm). If wavelengths greater than 320 nm are removed before phage irradiation,

decay rates are 2 to 10x higher (Suttle and Chen, 1992). Murray and Jackson (1993) found similar results that UV-B accounts for 50-90% of phage mortality. Also the distance that UV-B penetrates the water column depends upon the attenuation coefficient for the wavelength at a particular clarity of water. For example for 310 nm in clear ocean water, the attenuation coefficient is 0.15 m^{-1}, meaning that 22% of the surface irradiance will reaches 10 m. The same wavelength in moderate wave action has an attenuation coefficient of 0.86 m^{-1}, or only 2% reaches 10 m (Suttle and Chen, 1992).

The primary chromophore or photoreceptor for UV-B radiation is DNA, and the types of damage produced are the cyclobutane pyrimidine dimer and the pyrimidine (6-4) pyrimidone dimer. This is similar to the damage produced by UV-C radiation (Mitchell and Nairn, 1989). DNA absorbs approximately 0.1% of the radiation at a wavelength of 320 nm compared to the absorption of 260 nm (Jagger, 1985). At wavelengths above 300 nm, single-stranded breaks in the DNA and the formation of DNA-protein crosslinks become more significant (Mitchell and Nairn, 1989), but they are still not the major lesions. DNA dimers and single-strand breaks occur in a roughly one-one ratio in DNA exposed to 365 nm (Jagger, 1985). At wavelengths longer than 365 nm, DNA is not the main chromophore, but it is still the main target for damage, leading to the mutation and death of the cell (Eisenstark, 1989).

Pyrimidine dimers are the main lesions resulting from UV-B exposure demonstrated in *E. coli* bacteriophage ϕX174 (Patrick *et al.*, 1981). The 6-4

photoproduct accounts for approximately 10% of the UV damage in bacteriophage (Suttle, personal communication). Phage have two ways to repair UV damage: host dependent or host independent.

Host dependent repair mechanisms known for bacteriophage

Photoreactivation is the most studied host dependent mechanisms to repair bacteriophage DNA. Photoreactivation is an enzymatic repair system that absorbs a photon of light and the absorbed light provides the energy to break the covalent bond between the thymines in the DNA (Sancar, 1994). Ten to twenty photolyase molecules are estimated to exist in a single *E. coli* cell (Kim and Sancar, 1993). These enzymes find the dimer by random diffusion coupled with a $1x10^5$ discrimination ratio (specific binding coefficient/nonspecific binding coefficient). Once photolyase binds to the dimer, the dimer is split when a flavin adenine dinucleotide ($FADH^-$) in the enzyme absorbs a photon and then passes an electron to the dimer. Photolyase also contains cofactors for absorbing light in the near UV and visible spectrum where FADH absorbs weakly. These cofactors pass their photon energy onto the $FADH^-$ for dimer repair.

The first experimental results suggesting that the host cell's photoreactivation enzymes were responsible for rescuing bacteriophage damaged with UV-C were described by Dulbecco in the late 1940's, early 1950's (Dulbecco, 1949; 1950). However, not until the 1990's was the significance of this repair mechanism for

bacteriophage quantified in the environment. Curt Suttle's lab demonstrated that 67% of the native samples of bacteriophage for the marine bacterium *Vibrio natrigens* were repaired by light-dependent mechanisms (Weinbauer *et al.*, 1997). They discovered that exposure of bacteriophage to wavelengths between 370 and 550 nm restored infectivity.

A second type of host dependent repair is named the process of Weigle reactivation. Weigle reactivation was named after J. J. Weigle (1953) who first observed the phenomenon. The experimental method can be seen in Figure 1. It is a method in which host cells are induced to repair damaged viral DNA. What Weigle found from this experiment was that some phage were repaired better if the host cell had been irradiated just before or after infection with the damaged phage. Weigle's work was done with phage λ and various mutant strains of *E. coli* K12. Phages T2, T3 and T5 of *E. coli* did not Weigle reactivate under the same conditions as those used for λ. It was also observed that a high rate of mutant λ resulted from this process, suggesting that this was all controlled by the RecA protein and the induction of the *umuDC* genes, part of the SOS repair system (Walker, 1984). Further work in this area supported the idea that Weigle reactivation was under the control of the SOS repair system in that the protease activity of RecA is essential for Weigle reactivation (Tessman *et al.*, 1986). The LexA repressor must be cleaved to induce Weigle reactivation, but recombination activity of RecA is not necessary.

Figure 1. Experimental procedure for Weigle reactivation (Weigle 1953). A bacterial population is irradiated. UV-C inactivated bacteriophage are added to the irradiated bacteria and allowed to attach 15 min. The samples are plated by the soft agar overlay method and incubated in the dark or the light for 16 h. Plaque forming units (pfu) are enumerated and compared to non-irradiated bacteria.