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Full Length Research Paper

Comparison of the effects of UV-A radiation on *Leptospira interrogan* serovar Bataviae, Canicola and Pomona

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Motivated by the lack of related studies and an insufficient understanding of the response of pathogenic spirochetes, including leptospira to ultraviolet-A (UV-A) (or other stresses), we comparatively studied the effects of UV-A radiation on the *Leptospira interrogans* serovar Bataviae, Canicola and Pomona. The main purpose of this work was to investigate the effects of UV-A irradiation—both short term (immediate) and long term (post-irradiation)—on leptospires at different UV-A dosages, controlled by the duration of exposure time. It was observed that survival fractions linearly decrease from 100 to about 70, 60 and 50% for serovar Pomona, Bataviae and Canicola, respectively. This indicates that, for different serovars, UV-A irradiation has a quantitatively different effects on growth. Short term effects suggest that Pomona may be more resistant to UV-A than the other serovars. Long term effects show that, when compared with the control group, the treated groups of bacteria re-grow when the exposure time is equal or lesser than 6 h (~ 2 - 6), while the groups exposed for 12 h or longer experienced little change or a slight decrease. This may indicate that UV-A radiation is able to inhibit the growth of bacteria, but does not prevent self-defense from taking place. UV-A radiation's effect on antigenic components was also investigated. The immunoblotting method was used and the results are supported by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) results. Possible explanations for these results are discussed.

Key words: Ultraviolet-A, pathogenic spirochete, leptospirosis, *Leptospira interrogans.*

INTRODUCTION

It is well known that direct ultraviolet- A (UV-A) irradiation has detrimental effects of differing degree on bacteria cells depending on the nature of the radiation, on the type of bacteria and on the amount of the radiation (Ibanez et al. 2003; Jagger 1981). It is also known that sunlight is able to inactivate microorganisms due to the synergetic effects of its UV and infrared (IR) components (Rincon and Pulgarin, 2004). Since the spread of water borne infection is a problem encountered in both developed and underdeveloped countries (Labas et al., 2006), understanding the role that UV-A light plays in the disinfection process or in the photolysis process is needed, so as to improve various sanitation practices. Most studies on the effects of UV-A focus on *Escherichia coli* (Berney et al., 2006; Dantur and Pizarro, 2004). It is known that different micro-

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organisms respond differently to UV-A light (Berney et al., 2006; Oppezzo and Pizarro, 2001). But we must further know whether there are differences in the degree to which UV-A inactivates a particular microorganism and the degree to which the microorganism uses the enzyme photolysis to repair UV-A induced DNA lesions, which allows the microorganism to regain its viability.

Since pathogenic spirochete bacteria are another cause of disease when water pollution exists, the effects of UV-A on these bacteria must also be understood. In this paper, we are interested in the pathogenic spirochete bacteria of genus *Leptospira* that causes leptospirosis. This disease is a serious public health problem worldwide (American Public Health Association, 2000; Faine et al., 1999), as exemplified by recent large outbreaks in tropical and subtropical countries such as Nicaragua (Brandling-Bennett and Penheiro, 1996), Brazil, India (WHO, 2000), Thailand (Watt et al., 2003) and the United States (Centers for Disease Control and Prevention, 2000). Most outbreaks tend to be seasonal in nature and are often associated with environmental factors, animals and agricultural and occupational cycles, such as rice cultivation in marshy lands. The primary habitat of *Leptospira* is the mammalian kidney. When these leptospires are shed in urine from infected animals, they contaminate soil and water, thus creating transient foci for infection. The illness can range from being a mild flu-like illness to being a severe (often fatal) illness involving renal and/or liver failure and hemorrhaging (referred to as Weil's syndrome) (Sherris, 1984). More than 250 *Leptospira* serovars have been identified, which are classified into 24 serogroups (Turner, 1967). Some pathogenic *Leptospira* have been associated with domesticated animals. For example, the serovar Canicola has adapted itself to canines, so it has become common in many human communities. These pathogenic spirochetes may be affected by environmental factors such as magnetic field (Triampo et al., 2004), UV-A (Wong-ekkabut et al., 2009) and etc.

In this work, we compare the effects of UV-A radiation on three spirochete pathogens: *Leptospira interrogans* serovar Bataviae, Canicola and Pomona. The main purpose of our research was to investigate the effects of UV-A irradiation — both short term (immediate) and long term (post-irradiation) — on the leptospira at various UV-A dosages. Experimental leptospira growth and viability were determined by ultraviolet-visible (UV-VIS) spectrophotometry and dark-field microscopy. Changes in morphology and surface membrane were observed by scanning and transmission electron microscopy. Alteration of organism proteins or antigens was investigated by the immunoblotting method.

MATERIALS AND METHODS

UV-A irradiation source

In this experiment, UV-A radiation is generated by a 20 W T12 fluorescent lamp with a continuous emission spectrum of 320 - 400 nm and a peak of 365 nm. The UV-B and UV-C radiations are absorbed by the glass tube. The experimental samples were exposed to UV-A radiation at intensity of about 13 W/m^2. In Thailand, the average daily intensity of solar radiation is approximately 17.5 MJ/(m^2 day) and the temperature varies between 30 and 35°C, with a relative humidity between 50 and 80% on the average (Maneewan et al., 2004).

Strain and bacterial culture

Pathogenic *L. interrogans* serovar Bataviae, Canicola and Pomona were obtained from the National Institute of Health (NIH), Department of Medical Science, Ministry of Public Health, Thailand. They were grown in an Ellinghausen and McCullough liquid medium as modified by Johnson and Harris (EMJH) (Ellinghausen and McCullough, 1965), in the dark and at a temperature of 28 - 30°C. Leptospiral samples were sub-cultured at weekly intervals. Each 3 ml bacterial culture, in a 1.5 cm diameter glass tube, had an initial optical density (OD) of 400 nm ~ 0.15, as measured by a UV-VIS spectrophotometer (Schreier et al., 2009).

Irradiation procedure and experimental design

The pathogenic *L. interrogans* serovar Bataviae, Canicola and Pomona were cultured in EMJH medium until the logarithmic growth phase was achieved; then the solutions were diluted to OD ~0.15. The different suspensions were then exposed to UV-A radiation for various time with a light intensity of about 13 W/m^2. The set-up was organized as shown in Figure 1.

By adjusting the UV-A exposed durations, the selected dosages of UV-A treatment can replicate the daily exposure of UV-A in real life. The cultured bacteria were then exposed to UV-A radiation at variable time: 2, 6, 12 and 24 h, which corresponded to the radiation dosages 93.6, 280.80, 561.6 and 1123.2 kJ/m^2, respectively (radiation dosage = intensity x exposure time duration).

The data were obtained from two types of observations: short term effect (or immediate effect) and long term effect (or post-radiation effect). For the short term, the measurements were performed right after finishing the irradiation of the treated samples (survival fraction = OD of Control at t / OD of UV-A at t). For the long-term effect monitoring, all samples were sub-cultured right after the irradiation; then they were further cultivated under the same conditions as before (but with no treatment) for another seven days, similar measurements being performed. The experiments were repeated at least on three separate occasions (relative cell concentration = OD at tth day / OD at 0th day). The experimental procedure is diagrammatically summarized in Figure 2.

The leptospira were observed for growth, viability and morphological changes by using conventional dark field microscopy (DFM), UV-VIS spectrophotometry and electron microscopy. The changes of leptospiral proteins or antigens by experimental treatment were determined by the immunoblotting method, using antisera specific to individual leptospira serovars. The experiments were repeated at least three times under the same conditions.

Measurements

Quantitative analysis by UV-VIS spectroscopy for viability and survival

The cell density of leptospira was determined by UV-VIS spectrometer (Listgarten and Socransky, 1964; Tchamedeu et al., 2002). Leptospira cell suspensions were placed into a cuvette made with quartz SUPRASIL (200 - 2500 nm), with a light path of 10 mm. A

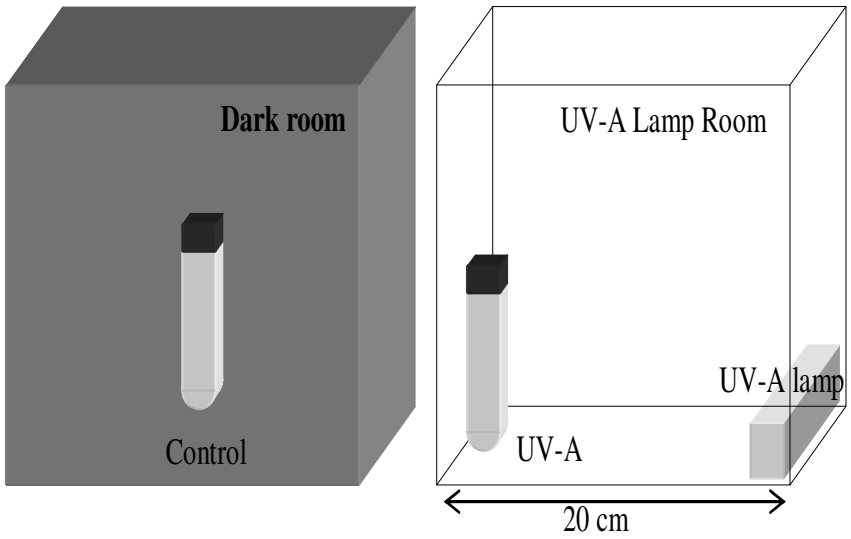

Figure 1. The schematic representation of experimental setup as described in texts.

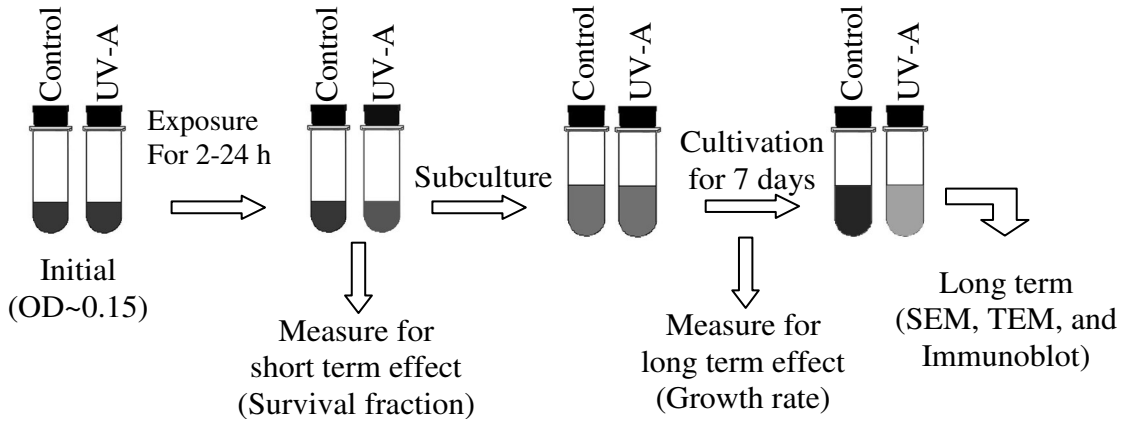

Figure 2. The schematic representation of the experimental procedure used in measuring short and long term effects.

single beam spectrometer operating in the range of 200 to 800 nm was used. The optical density or absorbency at 400 nm was taken (Jenkin et al., 1969; Oppezzo and Pizarro, 2001; Schreier et al., 2009) to characterize the growth corresponding to turbidity or the crowdedness of bacteria. All samples were evaluated with EMJH liquid medium as the blank. Cell counts of about 2.0 x 10^6 and 4.0 x 10^8/ml were equivalent to optical densities of 0.02 and 0.4, respectively. A difference of 0.04 in optical density is almost equivalent to a twofold difference in the cell count (Schreier et al., 2009).

Qualitative analysis by DFM

The growth and survival of the treated leptospira were determined by checking the morphology, density and mobility (Silva et al., 2001) of the organisms. In DFM, an oblique light beam is cast onto the leptospires (lying on a microscope slide) by the use of a special condenser, when the central illuminating light beam is interrupted. The leptospires can readily be seen as silvery threads in the dark background. To be able to clearly see an individual leptospira, the samples were diluted from 10-folds upward after exposure and re-subculture.

Qualitative analysis by scanning electron microscopy (SEM)

Samples were washed three times with normal saline (0.9% NaCl) at 10,000 X g for 10 min and were dropped on a poly L lysine-coated cover glass slide for 1 h. Samples were prefixed directly with 2.5% glutaraldehyde for 2 h at room temperature. After glutaraldehyde prefixation, samples were then washed three times with 0.01 M phosphate buffer (pH 7.3) for 10 min. All samples were post fixed with 0.1% osmium tetraoxide. After fixation, samples were dehydrated with increasing concentrations of ethanol, then critical point dried (HPC-2 critical point dryer Hitachi) and coated with a platinum-palladium ion sputter (E102 ion sputter, Hitachi) for 2 min. The micrographs were taken with an accelerating voltage of

15 kV SEM (S2500, Hitachi) on negative film.

Qualitative analysis by transmission electron microscopy (TEM)

For electron microscopic examination, the leptospires were concentrated by centrifugation (in a Micro 17R centrifuge at 10,000 RPM for 10 min) and prefixed with a 1.5% glutaraldehyde containing 0.1 M phosphate buffer, pH 7.2, at 4°C for 2 h, followed by postfix with a 1% osmium tetraoxide solution containing 0.1 M phosphate buffer at room temperature for 1 h and at subsequent steps as described (Thompson, 1986; Thompson and Manktelow, 1986). The samples were examined under a transmission electron microscope (TECNAI 20).

Immunoblotting for antigenic variation analysis

**Sodium dodecyl sulfate polyacrylamide gel electrophoresis (*SDS-PAGE) and immunoblotting*

The bacteria were washed with normal saline (0.9% NaCl) at 4°C three times for 5 min each and then lyzed with a standard Laemmli buffer composed of 62.5 mM Tris hydrochloride (pH 6.8), 10% glycerol, 5% 2-mercaptoethanol and 2% SDS and heated to 100°C for 5 min (Laemmli, 1970) and then boiled for 6 min. After removal of the remaining particulate materials by microcentrifugation at 10,000 × *g* for 5 min, the supernatant was loaded onto a 12% acrylamide gel. SDS-PAGE was performed in a Hoefer Mighty Small II mini-gel apparatus (Amersham Biosciences; San Francisco, CA, USA) using a constant voltage of 200 V for 1 h (Kelson et al., 1988). After completion, the resolved antigens were transferred onto a 0.45-µm-thick polyvinylidene fluoride (PVDF) membrane for 60 min, using a Semi-Dry system (TE70; Amersham Biosciences) with a constant current density of 1.5 mA/cm^2.

The blotted membrane was washed three times (5 min each) with 2% skimmed milk and 0.2 % tween20 (Sigma USA) in a phosphate buffer saline (PBS). For immunological detection, the membrane was incubated with a primary antibody (Rabbit reference antiserum against serovar Bataviae, Canicola and Pomona (Doungchawee et al., 2007) at 1: 1,000 dilutions in 2% skimmed milk and 0.2% tween20 in PBS for 1 h. The blotted membrane was washed three times for 10 min each with 2% skimmed milk and 0.2% tween20 in PBS. The membrane was transferred to a solution of secondary antibody (polyclonal goat anti rabbit immunoglobulin horseradish peroxidase (HPR) (DakoCytomation P0448) at 1: 2,000 in 2% skimmed milk and 0.2% tween20 in PBS, incubated for 1 h, and then washed three times for 5 min each in PBS. Color was developed with a solution of 1.25 mg of diaminobenzidine (DAB) and 5 µl of 35% H_2O_2 in 10 ml of PBS. The membrane was then rinsed with several changes of PBS to stop the reaction and air dried before being photographed.

The molecular weight protein standards (Amersham) used were rabbit muscle phosphorylase B (97 kDa), bovine serum albumin (66 kDa), hen egg white ovalbumin (45 kDa), bovine carbonic anhydrase (31 kDa), soybean trypsin inhibitor (20 kDa) and hen egg white lysozyme (14 kDa).

RESULTS AND DISCUSSION

The measurements were performed with an aim toward understanding the short term (or immediate) and the long term effects of UV-A radiation on the growth of these three serovars.

Short term effects

In Figure 3, it is seen that the effects of the UV-A radiation treatments on the three serovars (Bataviae, Canicola and Pomona) of pathogenic *Leptospira* are dose and serovar dependent for various dosages at 2, 6, 12 and 24 h. The responses of the different serovars are clearly different. We see in general that the decreases in cell viability are directly proportional to the exposure time. Survival fractions of these three serovars decrease linearly from 100 to 70, 60 and 50% for serovars Pomona, Bataviae and Canicola, respectively. After regression analyses were performed, it was found that the slopes (irradiation time-rate of decrease) of the survival fraction curves for the three serovars differed slightly from each other. The survival fraction slope of Ponona was the lowest (low rate of change), while those of Canicola and Bataviae were lower and similar to each other. Similar results were obtained in three independent experiments.

In Figure 4, DFM is shown to give a two dimensional visual impression of how the bacterial population changes as dose dependence. Under this magnification the morphological and topological changes of bacteria are not revealed, but the decrease in the number of cells, which is consistent with being measured by OD, is shown.

Long term effects

To investigate the long term effects, all the samples were sub-cultured and continuously cultivated for another 7 days. Here, we were interested in looking at whether the treated leptospires could survive and/or proliferate into the next generation.

Figure 5 shows the evolution of the bacteria populations of pathogenic *Leptospira* serovars Bataviae, Canicola and Pomona exposed to continuous illumination at 2, 6, 12 and 24 h. After samples were exposed to UV-A radiation, they were sub-cultured and re-cultured without radiation for 7 days. All studied *Leptospira* species behaved qualitatively in the same way. Monitoring of the bacterial concentrations in all three experimental leptospiral serovars showed decreases that were relative to exposure time. The concentrations of the leptospires exposed to irradiation for 6 h or less were found to increase, whereas no difference was observed among those exposed for longer durations (12 and 24 h). After 5 days of no irradiation, the new population of serovar Pomona and Canicola reached their maximum. It took longer for Bataviae to reach its maximum. Canicola had the highest survival fraction value (about 6). This might be due to differences in the experimental conditions or to fluctuations in the cultivation condition. By the 7th day, the survival fraction of all serovars had reached their maximums, which were different for each serovar. For Bataviae, the results were not conclusive since the populations of Bataviae are still

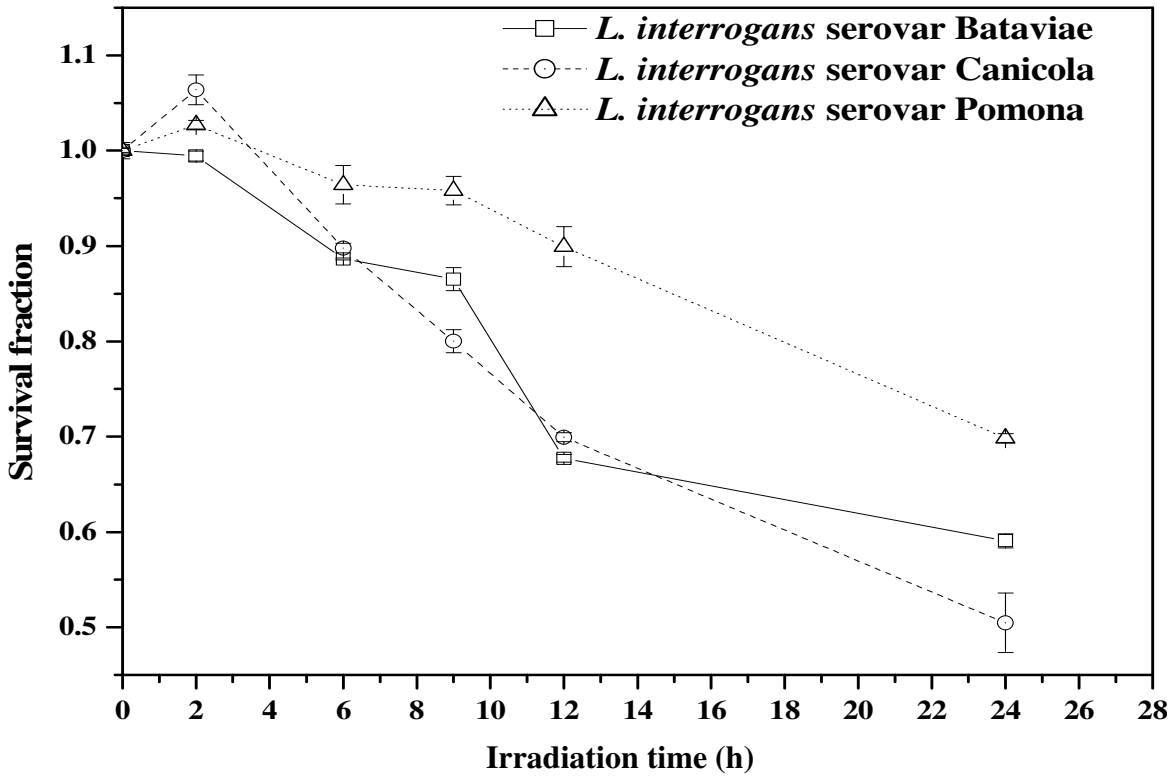

Figure 3. Survival graph of *L. interrogans* serovar Bataviae, Canicola and Pomona under UV-A irradiation, determined by an optical density (OD) measurement at 400 nm, with an initial leptospires count of 10^8cell/ml that decreases with irradiation time (survival fraction = OD of Control at t / OD of UV-A at t).

evolving. For 6 h or less of exposure, the exposed bacteria not only survived, but also continually multiplied until day 7.

Typical DFM of the leptospires taken on the 7th day for different irradiation time (from 2 - 24 h) are shown in Figure 6. The figures show that the bacteria concentrations varying from a dense state at low dose treatment to a very dilute state (and hard to find) at high dose treatment. In addition, a lot of spot-like objects are seen in the figures. We believe that these objects are the remains of the killed bacteria, as live spirochetes would be thread-like in shape. For the 24 h exposed bacteria, not only were the leptospires rarely observed; their mobility was very slow. This loss of mobility could be attributed to the denaturing of the periplasmic flagella.

Figure 7 shows the SEM micrographs of the leptospira. The control sample bacteria (the unexposed bacteria seen in Figure 7A) had wave-like shape, while the shape of the leptospira exposed to UV-A radiation for 24 h was deformed and had lost their wave-like shape.

The morphological differences between the unexposed and 24 h-exposed leptospires (after being sub-cultured for 7 days) is shown in Figure 8. The surface of the exposed bacteria appears rougher and may be floppier or bigger overall than the unexposed leptospires.

UV-A radiation's effect on the antigens of leptospires was examined by the immunoblotting method. Figure 9 shows the antigenic components of Bataviae and Canicola. Whole cell extracts were obtained from the UV-A exposed leptospires on the 7th day of sub-cultivation after radiation. Lane 1 is for the control sample (no exposure to UV-A radiation). Lanes 2 - 5 are for the samples which have been exposed to UV-A irradiation for 2, 6, 12 and 24 h, respectively. Comparing lane 5 with the control lane, we found that the antigenic components of Canicola with a molecular mass of 21 and 48 kDa had disappeared. These components are characterized to be part of the outer membrane (Cullen et al., 2002). The lanes for the serovar Bataviae also show similar changes; however, they are not as evident as the changes seen for Canicola. Since the serovar specific band of Pomona exists along the region of 20 - 21, 22 - 24 and 25 - 28 kDa (Doungchawee et al., 2007), it is difficult to observe whether this 21 kDa component of the tested Pomona changed.

Overall, our studies and samples demonstrate that Leptospires respond differently to UV-A irradiation treatments (in both the short term and long term) depending on the serovars and the applied doses.

The short time effects suggests that Pomona is more resistant to UV-A than Canicola and Bataviae. The different resistances to the effects of UV-A radiation of

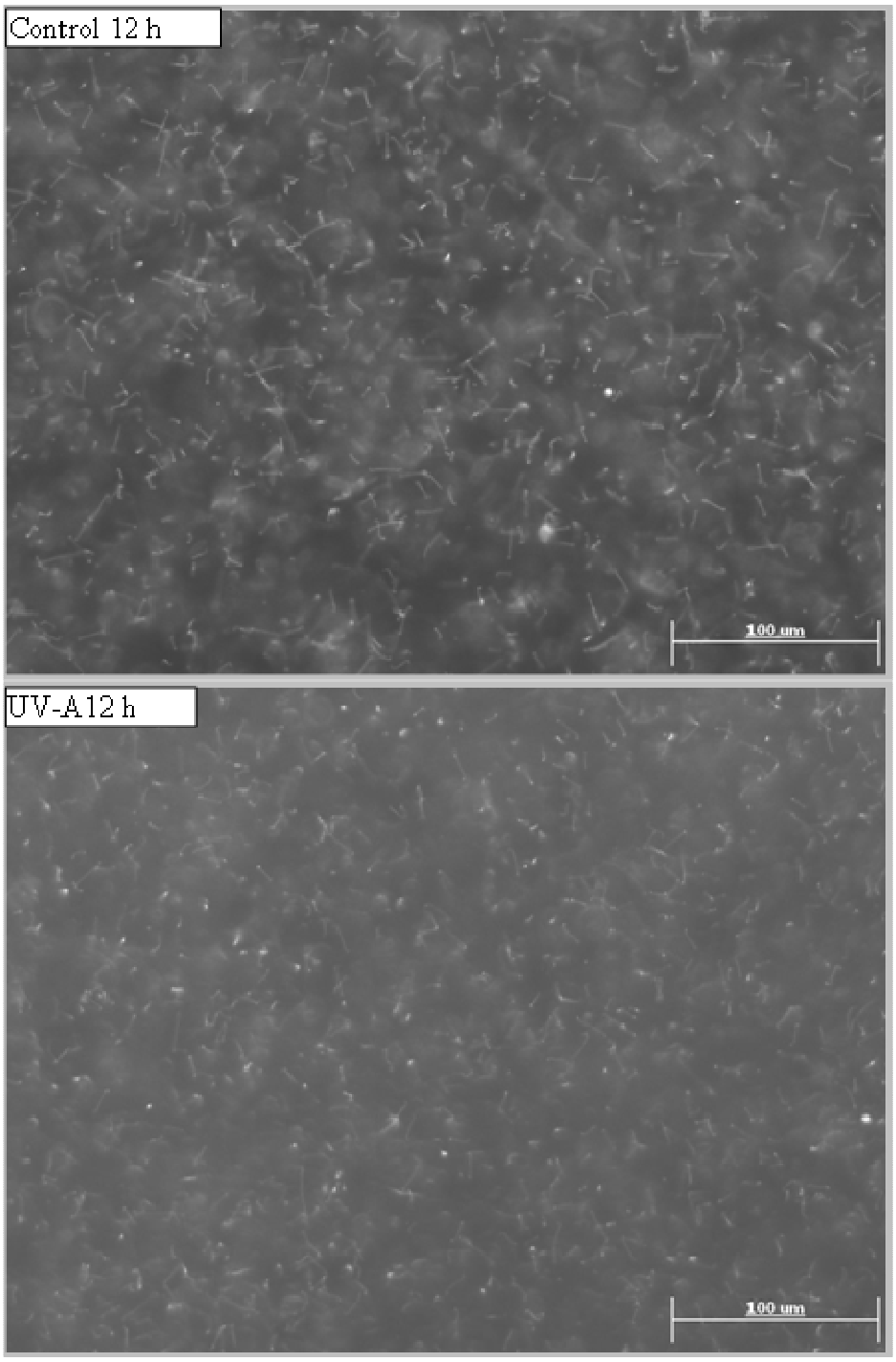

Figure 4. Dark-field optical micrograph (DFM) of *L. interrogans* serovar Bataviae represented as a small white thread-like structure taken 12 h after treatment with untreated control (above) and exposed to UV-A (lower). This image aims to quantitatively show the decrease of the bacterial population (magnification = x200, scale bar = 100 μm).

the different serovars may be due to an unknown mechanism of these leptospires. In addition, why and how the bacteria seem to vitalize in the first 2 h of exposure is still not understood. Intuitively, this finding may imply that certain serovars of leptospira are more sensitive to UV-A than others, as far as the growth response is concerned. This idea is apparently supported by the observations of Stamm and Charon (1988), whose

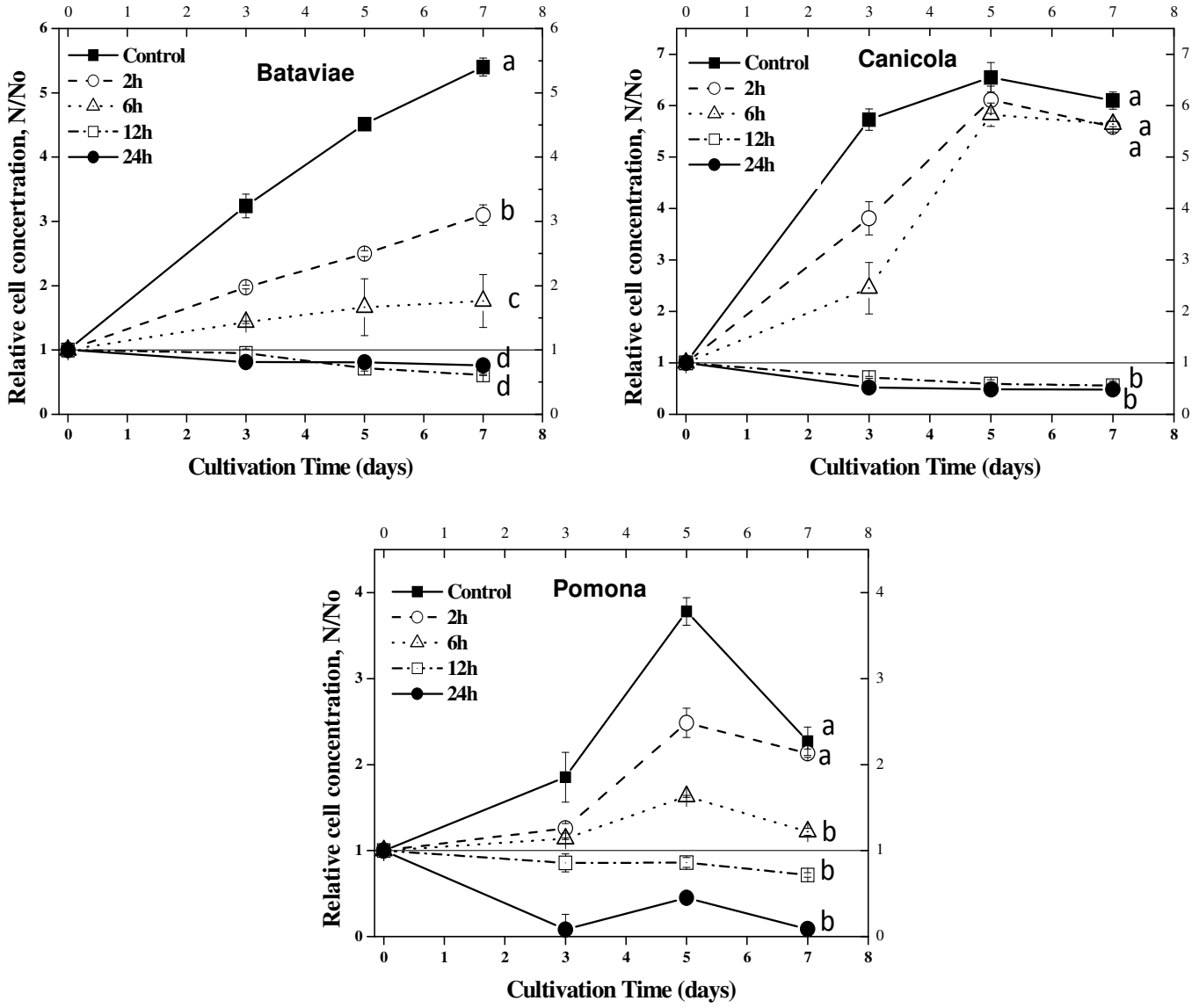

Figure 5. Growth curve of *L. interrogans* serovar Bataviae, Canicola and Pomona after exposure to UV-A for various time [0(control), 2, 6, 12 and 24 h]. The growth curves were determined after sub-culture and re-culture in the dark for 7 days (Relative cell concentration = OD at t^{th} day/ OD at 0^{th} day).

experimental evidence concluded that pathogenic *Leptospira* were more sensitive to UV radiation than were *Leptospira biflexa* serovars (non-pathogenic spirochete). When we compared the survival fractions of our three studied serovars with *E. coli* (Berney et al., 2006) and *Enterobacter cloacae* (Berney et al., 2006; Oppezzo and Pizarro, 2001) at a dose of about 1123.2kJ/m^2, all of them were relatively higher. This comparison may suggest that pathogenic *Leptospira* (at least for these three serovars) are more resistant to UV-A radiation than those bacteria. With regards to long term effects, when the treated groups of bacteria were compared with the curve of control group, they re-grew when the exposure time was equal or lesser than 6 h (~ 2 - 6), with a dose-dependent response, while those which were exposed for 12 h or longer were unchanged or slightly decreased. This indicates that some of the bacteria in the suspension, which had been injured by the photolytic treatment, were reactivated and re-grew after the irradiation was removed. In other words, the UV-A radiation was able to inhibit the growth of bacteria, but did not prevent self-defense from taking place, thus allowing the recovery and subsequent re-growth of bacteria. This finding is consistent with reports on similar studies on *E. coli* (Sommer et al., 1998) where it was demonstrated that the effectiveness of applying high UV intensity for a short time is greater than that

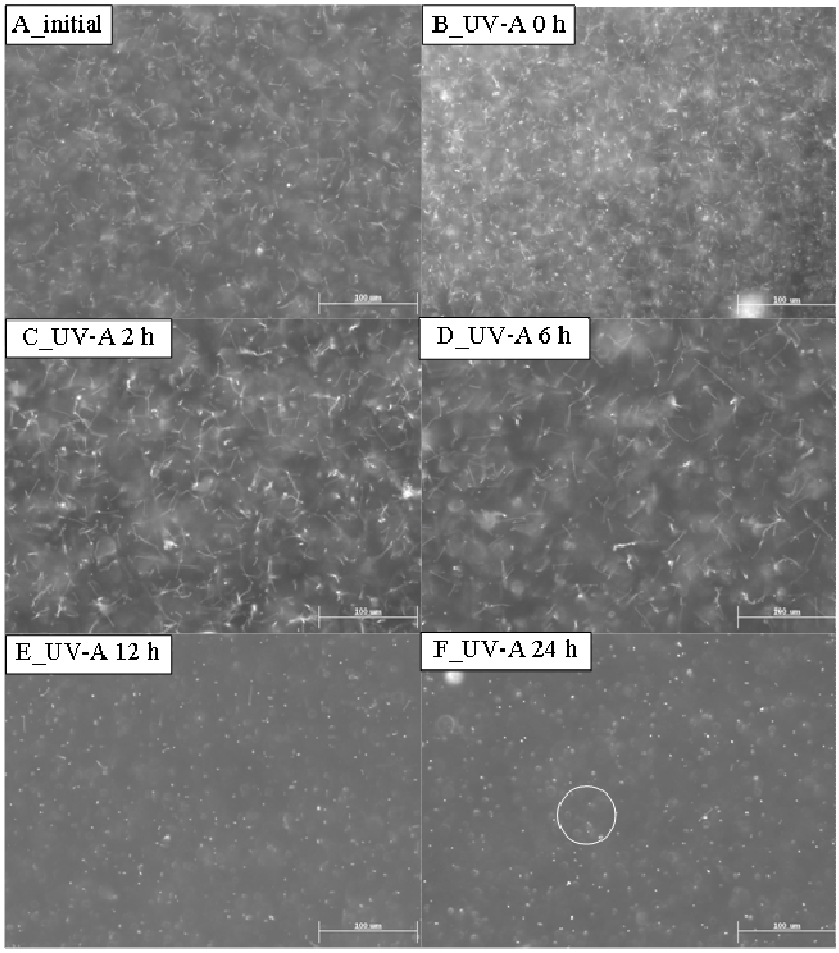

Figure 6. Dark-field optical micrograph (DFM) of *L. interrogans* serovar Bataviae at day 0 before sub-culturing (A) and after sub-culturing (B - F) for cultivation in the dark for 7 days, after UV-A irradiation for 0, 2, 6, 12 and 24 h, respectively (magnification = x200, scale bar = 100 μm).

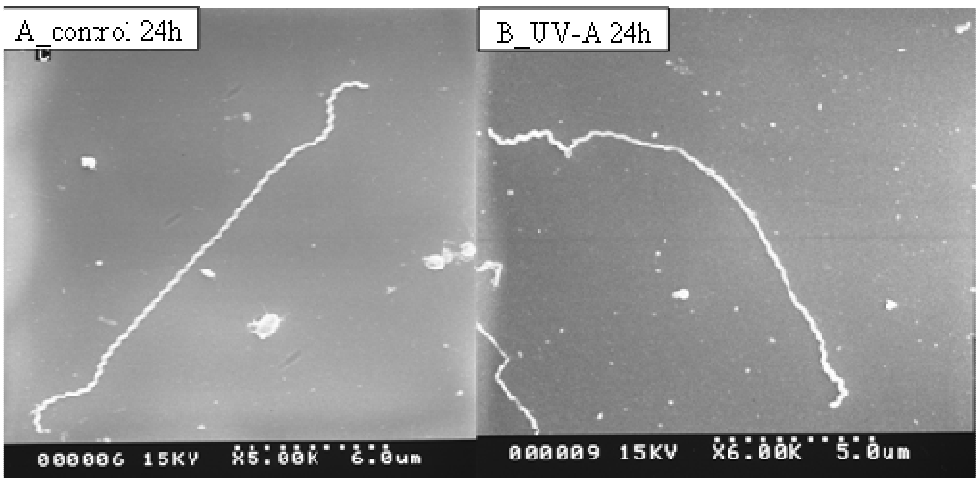

Figure 7. SEM micrographs of *L. interrogans* serovar Canicola control, unexposed to UV-A irradiation (left side) and after UV-A irradiation for 24 h and incubated for another 7 days (right side). Untreated control shows the typical wave-like shape of a typical leptospire, while the UV-A exposed sample shows an elongated and deformed structure.

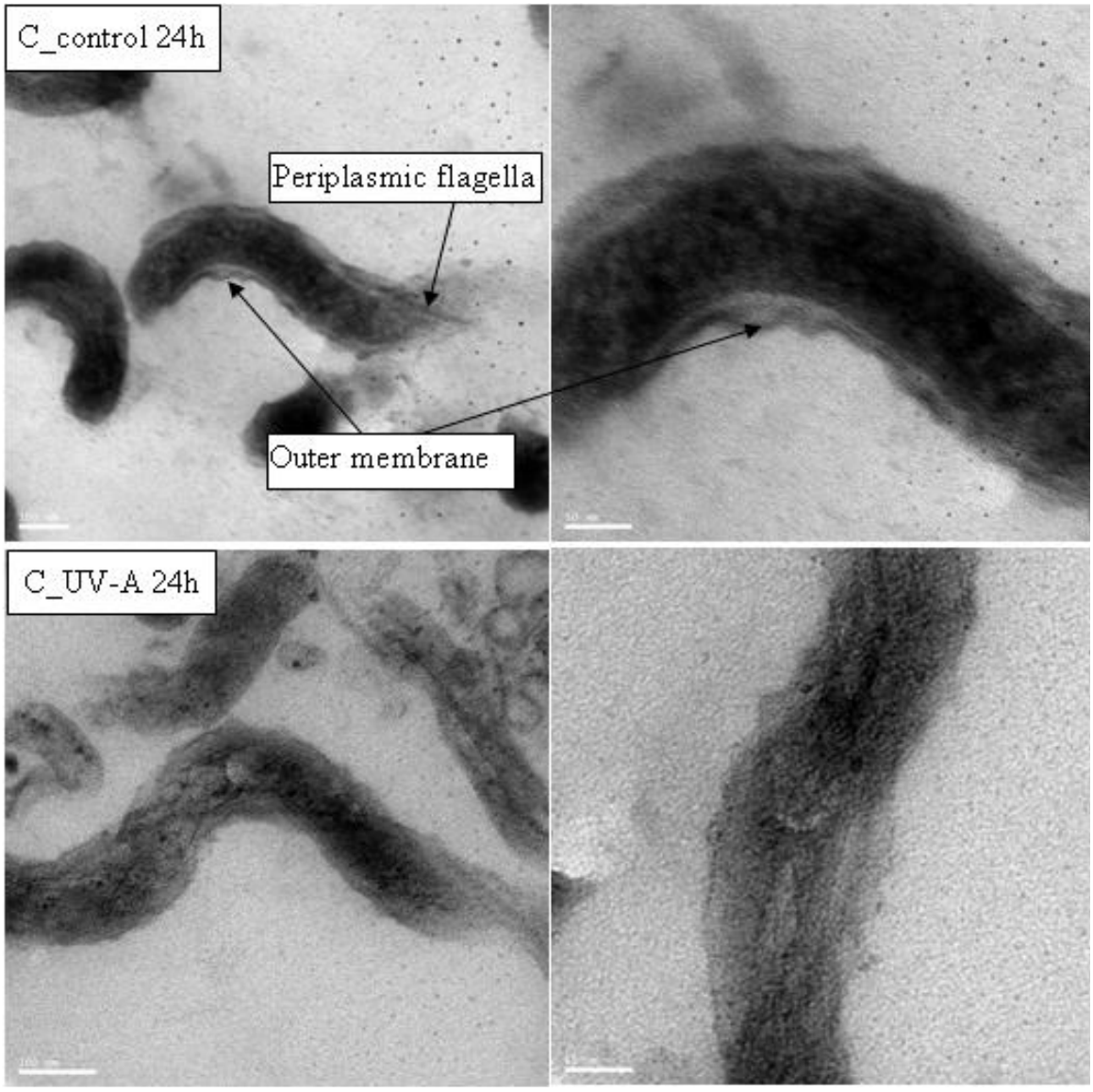

Figure 8. TEM micrographs of *L. interrogans* serovar Canicola control unexposed to UV-A irradiation (upper boxes) and after UV-A irradiation for 24 h and incubated for another 7 days (lower boxes). The control sample shows a typical normal outer membrane with axial filament, while the UV-A irradiated sample shows a significant degree of denaturation of the outer membrane with a noticeable rough surface.

when a lower intensity for a longer period of time is used. In addition, similar results of the sub-lethal UVA effect on *E. encloacae* in comparison with *E. coli* were reported by Oppezzo and Pizarro (2001).

Having worked on global analysis of outer membrane proteins from *L. interrogans* serovar Lai, Cullen et al. (2002) characterized these components as part of the outer membrane (Cullen et al., 2002). Though they used different serovar, it is reasonable enough to assume that it could be applied to other kinds of leptospira. They also indicated that the 21-kDa protein is the second most abundant constituent of the outer membrane proteins (OMP) of *Leptospira* serovar Lai and identified it as being lipL21 (3, 4). LipL21 is a surface-exposed, outer membrane (OM) lipoprotein that is expressed during infection and conserved in the pathogenic *Leptospira* species. In addition, Haake and Matsunaga (2002) identified the 48-kDa OMP as a novel OM lipoprotein designated as lipL48. Interestingly, Cullen and co-workers found that by applying thermal stress on pathogenic *Leptospira* serovar Lai, varying the temperature at 20, 30 and 37°C, the lipL48 was unaffected (Haake and Matsunaga, 2002). This finding suggests that 48-kDa could be damaged by UV-A radiation not by heat generation. Thus, UV-A radiation could induce outer membrane damage to the leptospira which could lead to a killing effect (Oppezzo and Pizarro,

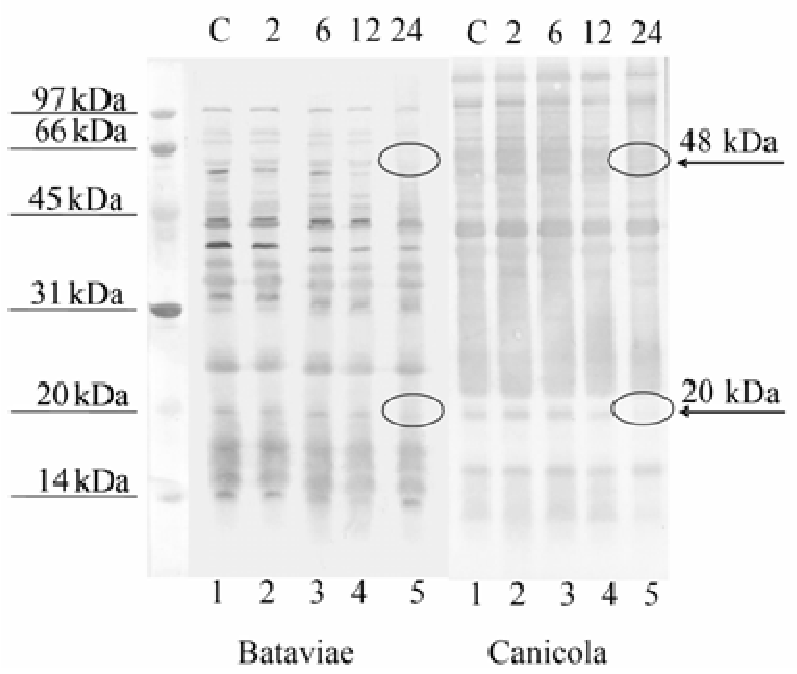

Figure 9. Immunoblot of serovar Bataviae and Canicola antigen extracted from cell after UV-A irradiation and incubated for another 7 days. Lane 1 is the control incubation in the dark; Lanes 2 - 5 are samples after UV-A irradiation for 2, 6, 12 and 24 h, respectively. Both extracted serovar antigens showed no significant change from their control at both 21 and 48 kDa, except for those samples treated for 24 h.

2001). Indeed, we have used electron microscopy techniques, TEM and SEM, to view the morphological changes (data not shown, see ref.) and saw the evidences of OM damage that may support these findings regarding OMP and/or lipopolysaccharide (LPS) denaturizing.

Conclusion

An understanding of the response of leptospira to UV-A (example, the survival rate and growth rate) is poor and needs to be developed. Our comparative studies of the response of pathogenic bacteria (*Leptospira* serovar Bataviae, Canicola and Pomona) to UV-A irradiation were performed in order to understand both short (or immediate) term effects and longer term effects, using several techniques: that is, growth and viability study techniques via OD, OM, SEM, TEM and immunoblotting.

Our studies show that UV-A irradiation causes short term effects to the survival fraction when the dosage is more than 93.6 kJ/m^2. The serovar Pomona seems to exhibit the largest resistance to UV-A radiation, while Canicola shows the least. As to long term effects, the 6 h or less exposed bacteria were not only able to survive, but also they were able to multiply after sub-culture and re-culture without UV-A radiation. The studies also show that for very high doses of UV-treatments (12 and 24 h), the self-defense and auto-repair mechanisms of bacteria are inefficient to protect cells. The exponential growth curve can not be recovered. Instead, the growth curves for the higher exposed bacteria show either constant or decaying behavior. This observation is supported by the DFM, SEM, TEM and antigenic electrophoresis data. Why these three serovars show some differences in their responses has not yet being studied. We believe that the differences are due to the differences in the structure and morphology of the outer membrane. Finally, we want to mention that this study shows that the inactivation behaviors due to UV-A radiation depend on the kind of bacteria being exposed. Increasing the doses increases the effectiveness of the inactivation.

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Abbreviations

SEM, Scanning electron microscopy; **TEM,** transmission electron microscopy; **UV-A,** ultraviolet-A; **IR,** infrared; **UV-VIS,** ultraviolet-visible; **OD,** optical density; **DFM,** dark field microscopy; **SDS-PAGE,** sodium dodecyl sulfate polyacrylamide gel electrophoresis; **PVDF,** polyvinylidene fluoride; **PBS,** phosphate buffer saline; **DAB,** diaminobenzidine; **HPR,** horseradish peroxidase; **OMP**, outer membrane proteins; **LPS,** lipopolysaccharide.

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