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Research Article

Effect of Chemical and Physical Treatment in Pseudomonas Aeruginosa ATCC 27853 and Bacillus subtilis ATCC 6633 Cells-Integrity

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Abstract

Several strategies have been proposed for the control of microbial pathogenic populations. Killing pathogenic bacteria present an international challenge that deserves particular attention to protect environment, Humain and animal health's. Fighting against pathogenic bacteria required a particular choice of adequate disinfection method. Mechanisms of microbial-cell inactivation of Gram-positive Bacillus subtilis ATCC 6633 and –negative bacteria Pseudomonas aeruginosa ATCC 27853 strains following three treatments ultrat violet C light (UV-C) -Sodium dodecyl sulphate (SDS)-phage (P) were considered. Results showed that UV-C up to 342 mWcm^{-2} dose discharged approximate 5-Log units of the viable and cultivable (VC) for *P. aeruginosa* ATCC 27853 strain. However, *B. subtilis* ATCC 6633 subjected to UV-C treatment resulted in 8-Log reductions of VC. Most of the *P. aeruginosa* ATCC 27853 cells inactivated by UV radiation remained unlysed in the absence of SDS or phage. SDS or Myovireadae phage Ω associated to UV treatment improve the disinfection with effect in cells integrity of both *B. subtilis* ATCC 6633 and *P. aeruginosa* ATCC 27853 strains. Bacterial lysis with chemical (SDS) or biological (phage Ω) amendments have the potential to improve cell death of irradiated bacteria.

Keywords: *Disinfection; P. aeruginosa; B. subtilis; Phage; SDS; UV-C*

Introduction

Over the world, infectious diseases are a significant burden on public-health and economic stability of all societies. Many methods have been well described in litterature as efficient tools for pathogenic bacteria destruction. These methods are classified as (i) chemical methods such as chlorination [1,2] and surfactant [3,4] (ii) physical approach for example UV-C irradiation, ultra-sonincation and micro-waves tools (iii) physico-chemical prossess such as polymer-based disinfectant [5,6], nano-photocatlytics and (iiii) biological methods and phage application [7,8]. Each method hold its proper advantages and disavantages. However, it exists a deep complementarity between some methods and when applied associated, the created system may improve water quality and may limit the spreading of some pathogenic bateria. Hybrid system may associate a serial of unit treatments having as a role to establish a complementarity between different treatment.

Ultra Violet germicide UV-C light radiation present a popular method in water treatement and was used as alternative to chemical disinfectants, because of the absence of by-products harmful to humans and the environment. Microbial disinfection by UV-C has the potential to reduce most enteric pathogenic bacteria [9-11]. The effect of UV-C on micro-organisms was discovered since 1877. Recently, UV-C effect in wastewater treatment deserves full attention of many works discovering its limits and its advantages [12,13]. However, some bacteria may escape to UV-C and may repair damage submitted to NAs, thanks to genetic patrimony. Most studies have been involved in UV-C and its effect in NAs bacteria damage and in bacteria NAs cells repair [14,15]. However, few studies have investigated the impact of UV-C radiation on bacteria cell integrity and behavior of cell function to damage. *B. subtilis* can protect itself by spore formation after stress irradiation [16,17]. However, *P. aeruginosa* repaired the cell damage by changes in NAs function following the administration of lethal dose of UV-C [18,19].

We wanted to know what happens to cell integrity after UV radiation. Could it be possible for the cells to repair and recover? To our knowledge the studies on bacterial cell integrity after UV-C irradiation were not established. Further lyses of these bacteria may be needed to achieve a full kill of pathogenic bacteria that have escaped the UV-C. The present work aims to expand upon previous work to exam the mechanisms of UV-C microbial cell inactivation by studying differences in Gram-positive and –negative bacteria and combinations of UV-C, SDS, or phage Ω isolated from domestic waste water.

Material and Methods

Pilot plant study

The batch laboratory UV-C device used in these studies was built in cooperation with Guy Daric S.A. (Aubervilliers, France). This prototype contained a sliding rack, with an irradiation board that held six Petri dishes (90 mm diameter). A germicidal low-pressure mercury vapour discharge lamp (length = 900 mm, diameter = 13 mm, power of UV emission at 253.7 nm = 55W) with reflector could be adjusted to different heights above the irradiation board to change relative dose zero to do 20000 mWscm^{-2}. An electric ballast supplied energy to the lamp and an extractor removed the ozone. Doses were calculated as the product of radiation intensity and time following the formula of Bolton, and Linden [20] equation 1.

UVDose = I·tR, (1) (equation 1)

UVDose expressed in (J/m^2), I (W/m^2) the irradiance (power delivered by the UV source), and tR (s) is the exposure time.

Microorganisms

P. aeruginosa ATCC 27853 and *B. subtilis* ATCC 6633 strains were kindly provided by Pr Boudabous Faculty of Sciences Tunis, Tunisia. Myovireadae phage Ω was isolated from non- treated domestic waste water sampled from the Pilot Plant Rades Tunisia and identified by Pr. Moineau Department of Medical Biology, Laval University, Quebec, Canada ax described by Grami., *et al*. [21].

Irradiation experiments

The irradiation studies used a batch laboratory irradiation device and followed the method of [12]. Cultures were inoculated at 1:200 dilution in nutrient broth (NB); Pasteur Institute Paris, France), from a fresh overnight culture grown at 37°C in water bath shaken at 220 rpm to midlog phase (OD600 0.4~2 X 10^6 cells ml-^{1}). *P. aeruginosa* and *B. subtilis* cultures were placed in 9-cm diameter glass Petri dishes and irradiated at increasing lengths of time with gentle agitation to obtain a calculated UV-C dose to a 3 mm depth of the solution. Two non-irradiated samples were also studied. At 15, 30 and 45 mn, 2 mL samples were taken from the flasks to count bacteria before (N_0) and after (N) exposure. Each experiment was repeated at least four times. Measurement of the incident intensity at 254 nm, at the liquid

surface, was measured using an ultraviolet Vilbert-Lourmat digital radiometerv France, Paris). Dose was expressed in mWscm-2 and was calculated as the average incident intensity. All irradiation was performed at laboratory temperature of 25°C ± 3°C. SDS was prepared at a stock concentration of 100 mM. Myovireadae phage Ω suspension at 107 PFU mL-1 was added to the cell suspension after cell irradiation. For all treatments, bacteria were counted by pour-plate technique on plate count Agar (PCA) medium (2 days at 37°C) according to French standards (Standard NF T 90-401).

Cell, nucleic acid and protein measurements

The UV-radiated cell suspensions were serially diluted with sterile 0.9% NaCl solution and spread on Luria-Bertani medium to enumerate *P. aeruginosa* ATCC 27853 and Nurtrient Broth medium to enumerate *B. subtilis* ATCC 6633 after incubation at 37°C for 24 h. Cell density was measured at 600 nm using a spectrophotometer method. The amount of protein released from the UV-C irradiated cells was measured by the method of [14,22]. Bovine serum albumin was used as the standard. The NA content of the suspension was directly measured at 260 nm using a UV spectrophotometer spectrophotometer (Perkin-Elmer, Model 560).

Statistical analysis

All experiments were repeated at least four times and data were subjected to analysis of variance and means were separated by the least significant difference according to the Student Newman-Keuls Test (SPSS 10 for windows).

Results

P. aeruginosa and *B. subtilis* strains were subjected to increasing UV-C irradiation doses, which reduced the VC. UV-C irradiation up to a dose of 342 mWcm-^{2} resulted in an approximate 5-Log units reduction of the VC in *P. aeruginosa* ATCC 27853 strain compared to initial counts (Figure 1). However, *B. subtilis* ATCC 6633 subjected to UV treatments as high as 842 mWcm-^{2} resulted in reductions of 8-Log VC.

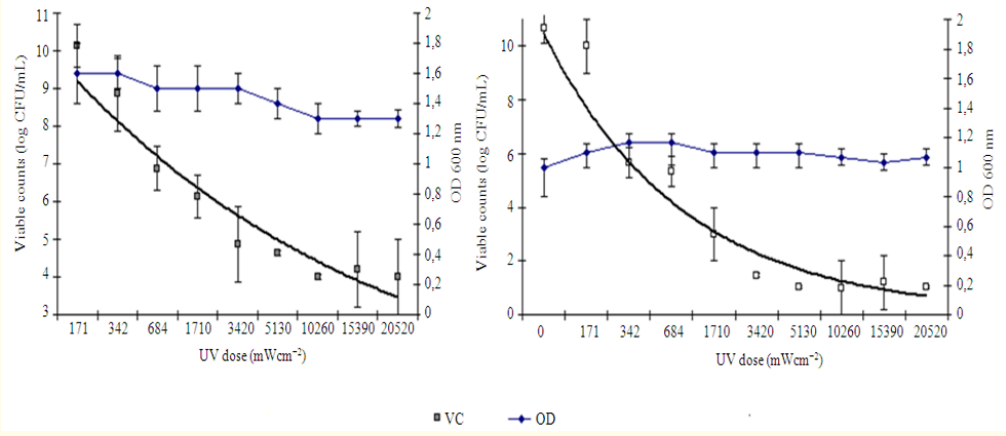

Figure 1: *Viable count and cell density of P. aeruginosa ATCC 27853 (A) and B. subtilis ATCC 6633 (B) before and after UV radiation at increasing UV doses considered in mWcm□2. Viable Count bacteria (VC) were counted by pour-plate technique on PCA me dium (2 days at 37°C) according to French standards (Standard NF T 90-401). DO = cells density determined by spectrophotometer method considered at 600 nm.*

Nucleic acids in suspension NAs

The amount of NAs released into the cell suspension was analyzed by measuring absorbance at 260 nm (Figure 2). The two bacterial strains tested showed similar Patterns in their release of NAs. However, the leakage of NAs from *B. subtilis* ATCC 6633 was higher than values found in *P. aeruginosa* ATCC 27853. The amount of released Protein RP into the cell suspension was also analyzed in both strains (Figure 2). Released Protein from *P. aeruginosa* showed an increase during the first time contact with UV-C light. In fact, the quantity of PR values varied from 300 to 365 µg/mL after 171 and 684 mWcm-^{2} radiation, respectively. After this first step, the RP showed a decrease to reach a value of 240 µg/mL after an exposition dose UV-C of 15390 mWcm-^{2}, respectively. However, *B. subtilis* showed an increase of RP related to an increase of UV doses and the amount of RP passed from 122 to 220 µg/mL when UV doses varied from 342 to 15280 mWcm-^{2}, respectively.

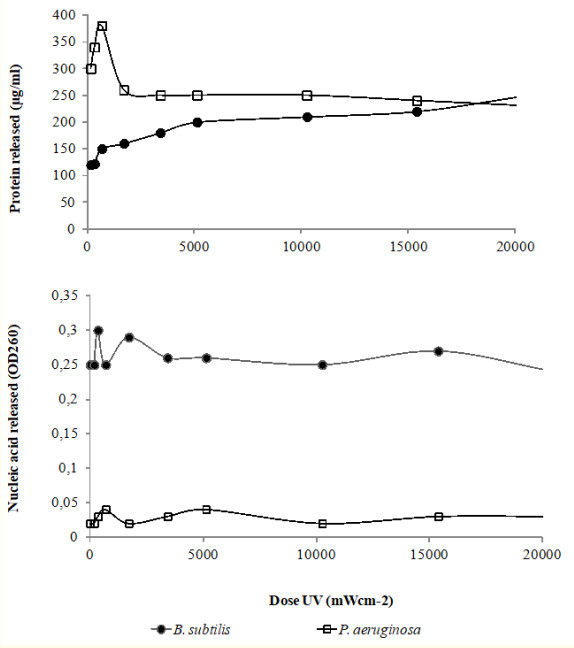

Figure 2: *Protein and nucleic acid released into the cell suspension of P. aeruginosa ATCC 27853 (A) and B. subtilis ATCC 6633 (B) at increasing UV doses considered in mWcm-2.*

Effect of UV-C treated cells to sds and myovireadae phage Ω

Results showed that the sensitivity of UV-C light to SDS lysis effect or to Myovireadae phage Ω varied with strain types (Figure 3). The density of UV-C radiated cell suspensions of *P. aeruginosa* ATCC 27853 was noticeably reduced within a half hour of incubation. The association of UV to SDS enhanced the reduction rate of *P. aeruginosa* growth illustrated by curve situated below the curve of UV no-associated to SDS. In addition, for *P. aeruginosa*, the Ω tested phage presented results similar to that one obtained when was used UV associated to SDS. However, in the case of *B. subtilis* the association of SDS to UV-C light did not ameliorate the destruction of cells expressed by curve situated above that of no associated UV-C to SDS curves.

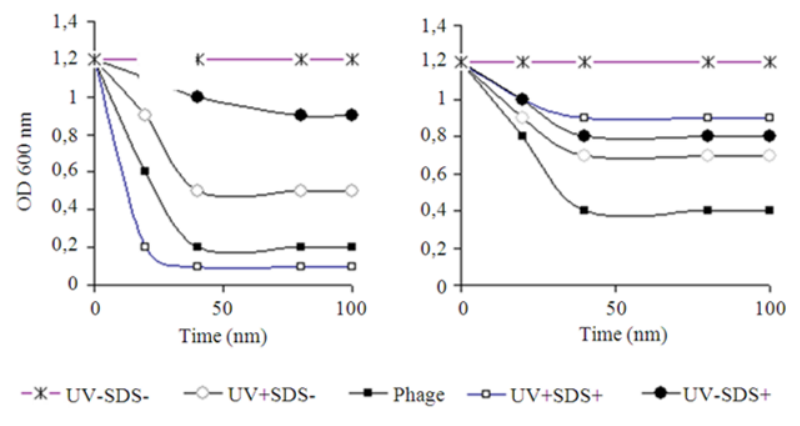

Figure 3: *The effect of UV radiation, SDS or phage treatment on cell density of P. aeruginosa ATCC 27853 (A) and B. subtilis ATCC 6633 (B) measured at 600 nm over 0 to 100 min incubation.*

Discussion

Lyses of nucleic acid in the suspension

Results showed clearly that *B. subtilis* ATCC 6633 was more sensitive to UV-C than *P. aeruginosa* ATCC 27853 at UV-C doses above 884 mWcm-^{2}. This result suggests that *B. subtilis* receiving UV-C light was submitted to membrane damage greater than *P. aeruginosa* [24]. It is known that Gram-negative peptidoglycan is only a few nanometers thick, representing one to a few layers. However, Gram-positive peptidoglycan is 30 - 100 nm thick and contains many layers [25]. This stronger cell wall layers supposed to confer to the Gram-positive bacteria greater resistance to stress. However, results obtained in the present study showed the opposite' and we showed that *P. aeruginosa* ATCC 27853 is more resistant to UV-C radiation than *B. subtilis*. Interestingly, it was observed that the cell density in both suspensions did not decrease in spite of significant reduction in the VC. This is because even if the cells are lysed, the lysed particles are still in the medium and they are also contributing to OD, hence Viable Cell Count is much more reliable method for viable cells determination which is clearly shown here. In addition, the constant value of OD may be attributed to that the UV treatment did not lyses the cells completely. The phenomenon of Viable Un Cultivable VUC state is an alarming case as a large number of organisms may go undetected in UV-C waters. The cells are VUC and undetec by routine culturing methods during this period. After UV-C treatment, this dormant state may be broken and cultivability may be regained, which may increase bacterial loads. During passage, in UV-C prototype, these bacteria may attain VUC state but viability will be achieved when bacteria will be released into natural conditions and proper nutrient availability. Thus, there is an increased chance of revival and proliferation of cells. The long survival and viability of these human pathogenic bacteria in seawater poses a serious concern for human health. Hence, it is imperative to follow water criteria sanitary before releasing it into the sea. Some authors recognized to combine two method of disinfection [23].

Nucleic acids release into suspension

Release of nucleic acids to suspension indicates damage to the cell membrane. Similarly, bacterial lyses also results in the release of intracellular proteins into the growth medium. The dose of UV treatment in *P. aeruginosa* ATCC 27853 affected the amount of protein

released into solution. A decrease in this amount was also observed as the UV doses increased. Up until a constant volume of NAs was released, the decrease in PR can be explained by the photo catalytic effect that UV will have on some protein molecules [26,27]. However, the amount of *B. subtilis* ATCC 6633 protein leaked in to solution increased with high UV doses. For *B. subtilis*, NAs released values in supernatant were bigger compared to NA released in the case of *P. aeruginosa*.

Effect of UV- SDS and myovireadae phage Ω in bacteria cells

The constant volume of NAs leaked into the solution and the decrease or increase of PR in solution by these two strains prompted us to use SDS and to correlate the effect of SDS on bacterial lyses. These results indicate that most of the *P. aeruginosa* ATCC 27853 cells inactivated by UV radiation remained unlisted in a cell suspension in the absence of SDS or phage. In addition cells were highly sensitive to lysis by SDS or by Myovireadae phage Ω. UV treated *B. subtilis* ATCC 6633 reacted differently to the treatments than did *P. aeruginosa* ATCC 27853. Bacterial lysis with chemical (SDS) or biological (phage Ω) amendments have the potential to improve cell death of irradiated bacteria.

Conclusion

Lyses of UVc treated cells may be realized via biological use of phage Ω or SDS and may improve the efficiency of UV-C disinfection. Thus, the UV-C light followed by SDS or phage Ω treatment may improve the sanitary quality of the disinfected water.

Bibliography

1. Chemingui H., *et al.* "Facile green synthesis of zinc oxide nanoparticles (ZnO NPs): Antibacterial and photocatalytic activities". *Materials Research Express* 6.10 (2019): 1050b4.
2. Zhang X., *et al.* "Formation and interdependence of disinfection byproducts during chlorination of natural organic matter in a conventional drinking water treatment plant". *Chemosphere* 242 (2020): 125227.
3. Basabe-Burgos O., *et al.* "Natural derived surfactant preparation as a carrier of polymyxin E for treatment of Pseudomonas aeruginosa pneumonia in a near-term rabbit model". *Journal of Aerosol Medicine and Pulmonary Drug Delivery* 32.2 (2019): 110-118.
4. Falk NA. "Surfactants as antimicrobials: A Brief overview of microbial interfacial chemistry and surfactant antimicrobial activity". *Journal of Surfactants and Detergents* 22.5 (2019): 1119-1127.
5. Ghernaout D., *et al.* "Disinfecting water: Electrocoagulation as an efficient process". *Applied Engineering* 3 (2019): 1-12.
6. Ounifi I., *et al.* "Synthesis and characterization of ultrafiltration membranes by phase inversion and by uropathogenic Escherichia coli retention performance". *Desalination and Water Treatment* 163 (2019): 109-117.
7. Edis Z and Bloukh SH. "Facile Synthesis of Antimicrobial Aloe Vera-"Smart" Triiodide-PVP Biomaterials". *Biomimetics* 5.3 (2020): 45.
8. Meile S., *et al.* "Reporter Phage-Based Detection of Bacterial Pathogens: Design Guidelines and Recent Developments". *Viruses* 12.9 (2020): 944.
9. Sichela C., *et al.* "Effect of UV solar intensity and dose on the photocatalytic disinfection of bacteria and fungi". *Catalysis Today* 129 (2007): 152-160.
10. Mutz YS., *et al.* "Modeling Salmonella Typhimurium inactivation in dry-fermented sausages: Previous habituation in the food matrix undermines UV-C decontamination efficacy". *Frontiers in Microbiology* 11 (2020): 591.

11. Tavares RD., *et al.* "Genotypic and phenotypic traits of blaCTX-M-carrying Escherichia coli strains from an UV-C-treated wastewater effluent". *Water Research* 184 (2020): 116079.

12. Saidi N., *et al.* "Biofilm and siderophore effects on secondary waste water disinfection". *Current Microbiology* 63 (2011): 337-340.

13. Rajkhowa S. "Heat, solar pasteurization, and ultraviolet radiation treatment for removal of waterborne pathogens". *In Waterborne Pathogens Butterworth-Heinemann* (2020): 169-187.

14. Memar MY., *et al.* "The central role of the SOS DNA repair system in antibiotics resistance: A new target for a new infectious treatment strategy". *Life Sciences* (2020): 118562.

15. Taylor W., *et al.* "DNA damage kills bacterial spores and cells exposed to 222-nanometer UV radiation". *Applied and Environmental Microbiology* 86.8 (2020).

16. Krystal R Kerney and Andrew C Schuerger. "Survival of Bacillus subtilis Endospores on ultraviolet-Irradiated rover wheels and mars regolith under simulated martian conditions Astrobiology Mary Ann Liebert, Inc. ry Ann Liebert, Inc 11.5 (2011).

17. Kogelheide F., *et al.* "The role of humidity and UV-C emission in the inactivation of B. subtilis spores during atmospheric-pressure dielectric barrier discharge treatment". *Journal of Physics D: Applied Physics* 53.29 (2020): 295201.

18. Slade D and Radman M. "Oxidative Stress Resistance in Deinococcus radiodurans Microbiol". *Microbiology and Molecular Biology Reviews* 75 (2011): 33-191.

19. Luján AM., *et al.* "ImuB and ImuC contribute to UV-induced mutagenesis as part of the SOS regulon in Pseudomonas aeruginosa". *Environmental and Molecules Must* 60.7 (2019): 594-601.

20. Bolton JR and Linden K. "Standardization of methods for fluence (UV dose) determination in bench-scale UV experiments". *Journal of Environmental Engineering* 129 (2003): 209-216.

21. Grami E., *et al.* "Siphoviridae bacteriophage treatment to reduce abundance and antibiotic resistance of Pseudomonas aeruginosa in wastewater". *International Journal of Environmental Science and Technology* (2021): 1-10.

22. Sawhney SK and Randhir S. "Introductory practical Biochemistry". *Narosa Publishing House* (2006): 64-262.

23. Ji-Hyoung H and SD Ha. "Synergistic effects of sodium hypochlorite and ultraviolet radiation in reducing the levels of selected food-borne pathogenic bacteria". *Foodborne Pathogens Disease* 8 (2011): 587-591.

24. Woo IS., *et al.* "Differential damage in bacterial cells by microwave radiation on the basis of cell wall structure". *Applied and Environmental Microbiology* 66.5 (2000): 2243-2247.

25. Silhavy TJ., *et al.* "The Bacterial Cell Envelope". *Cold Spring Harbor Perspectives in Biology* 2 (2010): a000414.

26. Neves-Petersen MT., *et al.* "UV light effects on proteins: from photochemistry to nanomedicine". *Molecular Photochemistry-Various Aspects* (2012): 125-158.

27. Gabriel MA., *et al.* "Ultraviolet irradiation of trypsin, lysozyme and β-galactosidase: how does UVC affect these enzymes when used as a secondary barrier against adventitious agents?" *Vaccine* 37.43 (2019): 6518-6525.

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