Original Article: Evaluation of the Effect of UV-C on Induction of Antibiotic Resistance of Bacillus Cereus and Bacillus Subtilis Bacteria in Laboratory Conditions

Valizadeh Behshad[*, a], HydarizadehYassin[b] ,Rezaie Mohamad Reza[b], Feghhi Seyed Amir Hossein [a]

[a]*Department of Radiation Application, Shahid Beheshti University, Tehran, Iran.*
[b]*Department of Nuclear Engineering, Faculty of Sciences and Modern Technologies, Graduate University of Advanced Technology, Kerman, Iran.*

Citation Valizadeh Behshad, HydarizadehYassin, Rezaie Mohamad Reza, Feghhi Seyed Amir Hossein. 2023. Evaluation of the Effect of UV-C on Induction of Antibiotic Resistance of Bacillus Cereus and Bacillus Subtilis Bacteria in Laboratory Conditions, *Journal of Research in Weed Science*, 6(1), 23-29.

http://dx.doi.org/10.26655/JRWEEDSCI.2023.6.3

Article info

Received: 2023.05.06
Accepted: 2023.05.24

Checked for Plagiarism:
Yes.

Peer reviewers approved by:
Dr. Mohammad Mehdizadeh

Editor who approved publication:
Dr. Amin Baghizadeh

***Corresponding Author:**
Valizadeh Behshad
(bvalizade0114@gmail.com)

Keywords:
Mueller Hinton Agar, Ultraviolet Radiation, Antibiotic, Antibiogram.

ABSTRACT

Bacterial infections have increased due to overuse of antibiotics and this issue has caused problems. One of the methods for destroy of infection is Ultraviolet radiation which can cause various behaviors in Bacteria and may eventually lead to genetic mutation .In this paper we study two types of bacteria such as *Bacillus cereus* and *Bacillus Subtilis* resistance before and after Ultraviolet radiation (UV-C). The susceptibility and resilience of bacteria to UV light were examined using four different kinds of antibiotics. Two strains were irradiated with Ultraviolet radiation and they were cultured in Mueller-Hinton Agar (MHA) and they were stored at 37^0 C and the growth of these two *Bacteria* was measured before and after irradiation. Irradiation was performed by using UV-C lamp. Antibiotic susceptibility of these two strains was investigated by using disk diffusion. Ultraviolet radiation and adding Antibiogram disks to the MHA culture caused *Bacteria* resistant to some disks. *Bacterial* colonies have become larger than before the ultraviolet radiation. According to the data, the number of bacteria declines as radiation exposure duration rises, suggesting that radiation could alter bacterial physiology.

Introduction

Bacterial resistance to drugs has seen abundance today. micro-organisms, like Bacteria may change after different times of radiation (Oskouee et al., 2020). The wavelength of non- ionizing ultraviolet radiation is various that divided in to UV-A, UV-B and UV-C, UV-A include(315-400nm), UV-B include(280-315nm) and UV-C include(100-280nm) (KASRA et al., 2005). Annihilation of micro-organisms depends on the dose of UV radiation. In the case the UV radiation be in touch with micro-organisms, there are the most effect and damage it may change, this radiation has the most effect (Mousavi, Golbabaei, Pourmand, Rezaei, Hosseini, Helmi Kohneh Shahri, et al., 2017). DNA damage and genetic mutation are all caused by UV radiation (Asgarani et al., 2006). micro-organisms can show various responses, including antibiotic resistance (Erfani et al., 2007). Bacillus Cereus and Bacillus Subtilis are gram- positive, aerobic, spores and saprophytes that are found everywhere (Molayi Kohneshahri

et al., 2015) (Pajohi et al., 2010).The coating on the spores makes the spores resistant to hard conditions(Ulrich et al., 2018). One of the agents with effect on food poisoning is Bacillus Cereus can produces toxic toxins that eventually lead to diarrhea and nausea, and most of these Bacteria are live in the soil (Yeo et al., 2011). The composed spore species can tolerance hard conditions such as high temperature and dryness (Pajohi et al., 2010).

Treatment with antibiotics should be done as needed because extreme use causes resistance(Kumar & Tripathi, 2017). Bacterial infections and inaccurate use of antibiotics have caused Bacterial show resistance to antibiotics (Norouzi et al., 2004). Micro-organisms show various sensitivities to radiation which depends on environmental agents (Kotiranta et al., 1999). The anti-oxidant compounds in the intra-cellular material of radiation resistant Bacteria have made them resistant to oxidative stress (Zamanian & Etemadifar, 2016). Recently, the abundant of resistance genes has reduced the performance of antibiotic (Berahou et al., 2007). Antibiotics can prevent Bacteria from reproduction and annihilate them (Kumar & Tripathi, 2017).

Resistance to Bacterial drugs causes a major challenge in healthcare field and infection and forces lots of heath costs. This study was performed to investigate the susceptibility form to UV radiation of these two strains that will be discussed in the following.

Methods

Collecting and identification of bacterial samples

This research is an experimental study that was performed by sampling from laboratory that has a radiation source. This sampling was performed to different areas: lamp key, lamp, latchkey, spring protection, door spring(inside), the floor and elevator. The sampling method was swab specimen that has contact with these areas. Swabs were cultured as sterile samples on MHA plates. Bacteria were identified by gram straining (Kiyani et al., 2017).

Antibiogram test

Antibiogram test is a quick way to determine the susceptibility of various micro-organisms to different antibiotics (Fatemi Motlagh et al., 2010) the diffusion disk method was used according to the CLSI guidelines to measure the resistance. Initially, the equivalent to half McFarland turbidity was prepared according to the protocol of MHA cultivation company (Erfani et al., 2008) and then the Bacterial suspension equivalent to half McFarland by the means swap, it was cultivated in a grassy manner on MHA culture (Saadat et al., 2014) and after that AZM(Azithromycin)-PRL (Piperacillin)-PB (Polymyxin-B)-OX (Oxacillin) disks (from medicine antibody company) were placed on the culture medium and using a sterile pence for do it.

Assessing the antibiotic susceptibility of strains to ultraviolet radiation

The samples were irradiated in two times (10 and 20 minutes) that the plates were opened in sterile condition during irradiation so whole plate was irradiated completely under radiation with 0/25 $^{J}/_{M^{-2}Sec^{-1}}$ intensity by UV lamp that oriented in distance of 40 cm from the radiation source. During irradiation, doors of case and control plates were open. The plates were directly under UV lamp. After irradiation, The samples were incubated for 18 hours at 37^0C then the inhibition zone diameter were measured using a growth measuring ruler (KASRA et al., 2005). Then by the means of a ruler, the inhibition zone diameter of bacterium was measured in millimeter and susceptibility changes were measured before and after irradiation.

Results and Discussion

The susceptibility to Bacteria can change and even annihilate over a period of time under radiation and can be resistant or susceptible depending on where they have already grown. The Bacillus cereus and Bacillus Subtilis grow after a first irradiation and their number after the second irradiation decrease. If the Bacteria are more resistant to antibiotics, they will live longer and if they are less resistant, they will die sooner

(Norouzi et al., 2004). These two strains are very important because of their spores and toxicity (Hosseini Doust et al., 2016). Spores can survive in difficult conditions and be present everywhere (Setlow, 2001). UV radiation, in addition to causing free radicals and damage to substances such as proteins and nucleic acids, as a result it disrupts and can lead to mutation (Ghafari, 2015). A study in 2017 showed the density of micro-organisms such as Bacillus Subtilis, Aspergillus Niger, Penicillin and Staphylococcus epidermis decreased after 60-90-120 minutes after UV radiation (Mousavi, Golbabaei, Pourmand, Rezaei, Hosseini, Helmi Kohneshahri, et al., 2017). Studies have shown that the resistance or susceptibility of Bacteria depends on their living environment; UV radiation can cause problems in these bacteria and depends on the irradiation time (Gayán et al., 2013). In 2020 an article evaluated the antibiotic susceptibility of resistant staphylococcus aureus Bacteria with six different antibiotics (Azithromycin- Piperacillin- Oxacillin- Trimethoprim- Rifampin- Polymyxin B) under the gamma radiation (300Gy). After irradiation (by gamma cell) it became resistant to Piperacillin and Polymyxin B and susceptible to Oxacillin and Rifampin and Azithromycin (Oskouee et al., 2020). The results of this research are consistent with the results of Tamizi et al (2017) with the difference that in this article different doses of ultraviolet radiation were investigated.

Study of mutated Bacteria with two types of UV and gamma radiation was shown the resistant to Arsenic toxicity. The results showed within 3 minutes of gamma irradiation and 120 minutes of UV irradiation, in addition to Bacterial resistance, the bacterial population were increased compared to the prototype (shafaei et al., 2017). In 2015, Yelmeh et al. Investigated the effect of UV radiation on the Escherichia Coli and Bacillus cereus Bacteria in milk and rice, which result shown Bacillus cereus is more resistant and the growth and number of Bacteria decreased (Yolmeh et al., 2015). This result are consistent with our results and after ultraviolet irradiation, the number of Bacteria were decreased by increasing irradiation. In 2010, Marko et al. Studied Bacillus Subtilis Bacterium under ultraviolet radiation that Bacterial population have increased compared to bacteria have not exposed to UV radiation (control group) (Wassmann et al., 2010).

Table 1 shows the results of the susceptibility and resistance of Bacillus Subtilic and Bacillus Cereus before and after irradiation versus millimeters on Bacteria. In figure 1 for Bacillus Cereus It was not formed any zone for PB and OX in control group and after UV they became susceptible. PRL and AZM after irradiation they were susceptible than control group. OX had mobility after irradiation and formed two zones. PRL has mobility before irradiation and after UV radiation the mobility has decreased. AZM forms two zones before irradiation with small colonies and colonies disappear after irradiation. For Bacillus Subtilis in figure 1 it was found that the susceptibility to OX, PB and PRL after UV radiation was decreased and the susceptibility for AZM after irradiation was increased. OX has small colonies before irradiation and after irradiation the number of colonies increased and also had mobility. The mobility of PB after irradiation has lost and is closer to the disk. PRL protect the mobility before and after irradiation but the mobility after radiation was decreased. AZM formed two zones after the irradiation although in control group there was only one zone. In figure 3 Bacteria colonies have also become larger and their growth increase than before ultraviolet radiation. In control group (A), the colonies are very small than the others and after irradiation the small colonies have changed as figures 3-B and 3-C. But after 20 minutes the number of colonies was decreased.

Table1. The inhibition zone diameter before and after UV

Antibiotic	Before UV(Control group)(mm)		After UV(mm)	
	Sample MHA1	Sample MHA2	Sample MHA1	Sample MHA2
AZM	35	24	30	1= 35, 2=30
OX	-	20	14	1=16,2=8
PRL	29	20	25	18
PB	-	6	10	-

**first sample is Bacillus Cereus, second sample is Bacillus Subtilis, 1=large colonies, 2=small colonies*

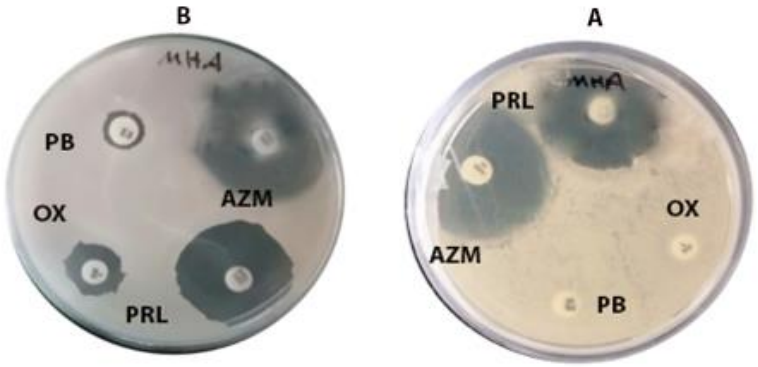

Figure1. *Bacillus* Cereus *Bacterium* before UV irradiation and after 10 minutes' irradiation, A) before irradiation (control group) that is cultured in MHA plates and B) the antibiogram test after 10 minutes irradiation

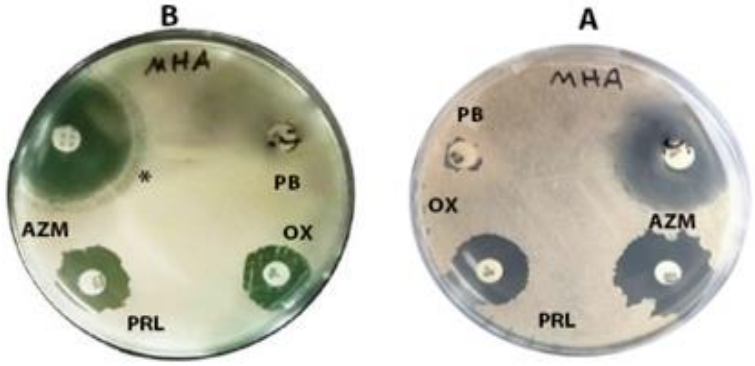

Figure2.*Bacillus* subtilis *Bacterium* test before and after UV irradiation, A) the control group B) after UV irradiation. (MHA culture), *= area of two zones

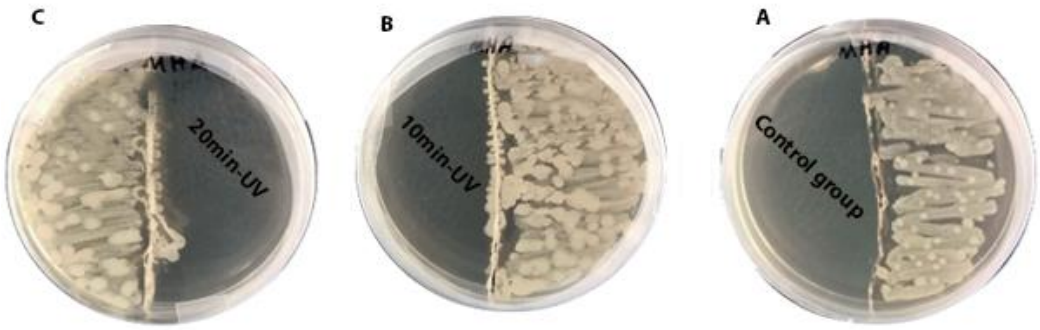

Figure3. A) Control group, B) 10 minutes' test C) 20 minutes' test. MHA culture (MHA with the density of half McFarland) as it seen the *Bacterial* colonies are small in control group and after irradiation, dimensions of colonies were changed and became larger

Conclusions

This study was performed to investigate the effect of UV-C radiation on induction of antibiotic resistance of *Bacillus Cereus* and *Bacillus Subtilis Bacteria.* Ultraviolet radiation showed the susceptibility to OX and PB disk in *Bacillus Cereus* were increased and the susceptibility to PRL, OX and PB disk in *Bacillus Subtilis* were decreased and this is in turn promotes the spread and resistance to antibiotic. The present study showed that Bacteria behave differently against radiations. It is probability that the Bacteria were previously exposed to gamma radiation (Cs-137 source - 100 mCi), in fact the Bacteria have been irradiated twice(UV irradiation after gamma irradiation). They were first exposed to gamma radiation and then exposed to UV radiation. As a result, radiation resistance can indirectly transfer resistance to antibiotics and due to expression in the environment, the strain can have speared and resistance. The innovation of this article is that it has investigated the change in the behavior of bacteria due to gamma and ultraviolet radiation. First, the bacterial samples were irradiated by gamma radiation and then they were irradiated to ultraviolet radiation to investigate the change in their behavior.

Author Contributions

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Behshad Valizadeh, Mohammad Reza Rezaie, Yassin Heidarizadeh ,Seyed Amir Hossein Feghhi .The first draft of the manuscript was written by Seyed Amir Hossein Feghhi and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that they have no conflicting interests.

Availability of data and material

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Ethical approval

This article does not contain any studies with human subjects or animals performed by any of the above authors.

References

Asgarni E, Shirzad M, Soudi MR, Shahmohammadi HR, Falsafi T. 2006. Study on the resistance of haloferax radiotolerans, an extreme Halophilic archaebacterium from Uromia lake against ultraviolet (UV) light and 60 Co gamma-rays. *J Nucl Sci Techno.*13-18 , [Google Scholar], [Publisher]

Berahou A, Auhmani A, Fdil N, Benharref A, Jana M, Gadhi CA. 2007. Antibacterial activity of Quercus ilex bark's extracts. *J*

Ethnopharmacol.112: 426-429 [Crossref], [Google Scholar], [Publisher]

Erfani Y, Safdari R, Chobineh H, Mir Salehian A, Rasti A, Eynollahi N, Mir Afshar SM, Yazdanbod H, Hamidian M, Soltanian A. 2008. Comparison of E. test and disk diffusion agar in detection of antibiotic susceptibility of E. coli isolated from patients with urinary tract infection in Tehran Shariati Hospital. Avicenna *J Clin Med* 15:27-31. [Google Scholar], [Publisher]

Erfani Y, Safdari R, Rasti A, Sharifi Yazdi MK, Jahanmehr SA, Yazdanbod H, Sadeghi F. 2007. Evaluation of Antibiotic Susceptibility of Enterobacteriacea Isolates from Blood Cultures. *Payavard Salamat*. 1:17-23. [Google Scholar], [Publisher]

Fatemi Motlagh M, Varham H, Mansori N. 2010. Gradient Plate for Hicomb Minimum Inhibitory Concentration (MIC) Test. *Med Lab J* 4: 45-51. [Google Scholar], [Publisher]

Gayán E, Álvarez I, Condón S. 2013. Inactivation of bacterial spores by UV-C light. *Innov Food Sci Emerg Technol* 19: 140-145 [Crossref], [Google Scholar], [Publisher]

Ghafari MD. 2015. Cloning and expression of Deinococcus radiodurans drRRA gene in E. coli Origami strain. *New Cellular and Molecular Biotechnology Journal*5:94-89. [Google Scholar], [Publisher]

Hosseini Doust R, Akhgari M, Ketabi H. 2016. Identification of Microbial (Bacillus spp.) Contamination in Illicit Drugs. *Iranian Journal of Forensic Medicine*21: 207-213. [Google Scholar], [Publisher]

Kasra Kermanshahi R, Heshmat Dehkordi E, Kazemi M. 2005. Effectiveness of Gamma Ray and UV Light on the Penecillin Resistant Strains of Staphylococcus aureus. *Avicenna Journal of Clinical Medicine* 12:44-50 [Google Scholar], [Publisher]

Kiyani L, Rezatofighi SE, Motamedi H. 2017. Identification and study of antibiotic resistance of bacteria in membranes of household water filter systems in Ahvaz. *Iranian Journal of Health and Environment* 9: 559-570. [Google Scholar], [Publisher]

Kotiranta AK, Ito H, Haapasalo MP, Lounatmaa K. 1999. Radiation sensitivity of Bacillus cereus with and without a crystalline surface protein layer.*FEMS Microbiol Lett*. 179: 275-280 [Crossref], [Google Scholar], [Publisher]

Kumar S, Tripathi M. 2017. Antibiotic resistant bacteria: A global menace. *Virol Immunol J* 1: 1-3. [Google Scholar], [Publisher]

Molayi Kohneshahri S, Taghinejad J, Hosainzadegan H. 2015. A review on structure and virulence of Bacillus cereus. *Laboratory & Diagnosis*. 7: 51-67. [Google Scholar], [Publisher]

Mousavi T, Golbabaei F, Pourmand MR, Rezaei S, Hosseini M, Helmi Kohneshahri M, Masoorian E, Karimi A. 2017. Evaluating the efficiency of UVC radiation on HEPA filters to remove airborne microorganisms. *J Saf Health Work*. 7: 111-120. [Google Scholar], [Publisher]

Mousavi T, Golbabaei F, Pourmand MR, Rezaei S, Hosseini M, Helmi Kohneshahri M, Masoorian E, Karimi A. 2017. Evaluating the efficiency of UVC radiation on HEPA filters to remove airborne microorganisms. *J Saf Health Work*. 7: 111-120. [Google Scholar], [Publisher]

Norouzi J, Vali GR, Yousefi H. 2004. Surveying the effects of different methods of mutations on the antibiotic resistance patterns and plasmids in E. Coli and Staph. Aureus. *KAUMS Journal (FEYZ)* 8: 1-8. [Google Scholar], [Publisher]

Oskouee S, Feghhi SA, Soleimani N. 2020. Antibiotic susceptibility variations of Methicillin-resistant Staphylococcus aureus after gamma irradiation. *Int J Radiat Biol* 96: 390-393. [Crossref], [Google Scholar], [Publisher]

Pajohi M, Tajik H, Farshid A. 2010.Antimicrobial activity of Nisin on Bacillus cereus and Bacillus subtilis in a food model and their ultra structural investigation. *Iran J Med Microbiol* 4: 45-52. [Google Scholar], [Publisher]

Saadat S, Solhjoo K, Norouz-nejadfard MJ, Kazemi A, Rouhi R, Mardaneh J. 2014.The frequency of Staphylococcus aureus among Shiraz hospital personnel and determination of their antibiotic sensitivity pattern. Iran South Med J 17(5), 916-926. [Google Scholar], [Publisher]

Setlow P. 2001. Resistance of spores of Bacillus species to ultraviolet light. *Environ Mol Mutagen* 38(2-3):97-104 [Crossref], [Google Scholar], [Publisher]

Shafaei M, Tamizi Z, Moshtaghiun M. 2017. Comparison of the effects of ultraviolet and gamma rays on arsenic tolerance in the bioleaching process. *Radiation Safety and Measurement* 6: 1-6. [Google Scholar], [Publisher]

Ulrich N, Nagler K, Laue M, Cockell CS, Setlow P, Moeller R. 2018. Experimental studies addressing the longevity of Bacillus subtilis spores–The first data from a 500-year experiment. *PLoS One*. 13: e0208425 [Crossref], [Google Scholar], [Publisher]

Wassmann M, Moeller R, Reitz G, Rettberg P. 2010. Adaptation of Bacillus subtilis cells to Archean-like UV climate: relevant hints of microbial evolution to remarkably increased radiation resistance. Astrobiology. 10: 605-615 [Crossref], [Google Scholar], [Publisher]

Yeo IC, Lee NK, Cha CJ, Hahm YT. 2011;.Narrow antagonistic activity of antimicrobial peptide from Bacillus subtilis SCK-2 against Bacillus cereus. *J Biosci Bioeng*. 112: 338-344 [Crossref], [Google Scholar], [Publisher]

Yolmeh M, Habibi-Najafi MB, Najafzadeh M. 2015. Study the effects of ultraviolet radiation on the growth of Escherichia coli and Bacillus cereus isolated from raw milk and raw rice. *Iranian Food Science and Technology Research Journal.* 11: 319 [Google Scholar], [Publisher]

Zamanian N, Etemadifar Z. 2016. Isolation and molecular identification of a UV-resistant strain of Dietzia maris and antioxidant activity of pigment. *Zistshinasii Mikruurganismha* 5: 83-94 [Crossref], [Google Scholar], [Publisher]