RESEARCH ARTICLE

Effect of Different Laser Irradiation Parameters on the Viability of Bacillus Species

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ABSTRACT

The genus *Bacillus* is present everywhere in the environment, it has environmental, industrial, and medical significance. This heterogeneous group of bacteria shares unique characteristics, one of these is the resistance to physical and chemical agents. Laser light is one of the most interesting physical agents with a wide range of applications in all scientific fields. It has the ability to alter bacterial growth by triggering inhibitory or stimulatory mechanisms. In this study, laser parameters were varied to evaluate the effect of each one on the viability of *Bacillus* species. CW or chopped (532 nm) laser light at different irradiation periods were examined. Results showed that tested bacterial species' response to green laser light varied. Bacterial viability was dropped with increasing chopping frequency and exposure time. In conclusion, high repetition rate and prolonged exposure time increase the lethal effects of this type of laser.

Keywords: Green laser, *Bacillus*, Low-level laser, Cytotoxicity, Bactericidal effect.

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INTRODUCTION

The genus *Bacillus* is everywhere in the environment, from soil to aquatic systems and the air. Members of this genus are gram-positive rods, endospore-forming, which occur singly or in chains.[1] *Bacillus* species show a wide range of characteristics and diversity related to their large genetic variations that allow them to live and adapt in most environments. Spores formed by *Bacillus* species resist heat, dehydration, and ionizing radiation.[2] *Bacillus* species have both medical and industrial importance since some *Bacillus* members cause severe diseases for animals and others cause food poisoning as well as food spoilage.[3] *Bacillus* spp. are of special importance for many biotechnological applications, such as probiotic dietary supplements, production of antibiotics, and genetic engineering [4]there is limited information on their resistance to various antibiotics, and there is a growing concern over the transfer of antibiotic resistance genes. The MIC for eight antibiotics was determined for 85 Bacillus species strains, Bacillus subtilis subsp. subtilis (n = 29).

Laser light is one of the most interesting physical agents that has a wide range of applications in approximately all scientific fields.[5] Low-level visible laser has the ability to stimulate intracellular processes resulting in distinct biological effects.[6] Visible laser light at higher intensities was found to have bactericidal effects while at lower intensities, may enhances bacterial proliferation.[7] Recent studies have shown that the short-term red laser treatment of *Bacillus* subtilis can increase their growth and activity. This effect can be used to increase the productivity of this bacterium.[8]

In this study, we have a focus on the possible lethal effects of green laser light on *Bacillus* spp. using different laser parameters. In addition, the study attempts to elucidate the variation in responses among different species.

MATERIALS AND METHODS

Bacteria

Bacterial isolates (*B. cereus, B. licheniformis, B. subtilis* and *B. coagulans*) were obtained from the soil samples which were collected from different places in Baghdad. After inhibiting other types of non-spore forming bacteria by heating, suspected *Bacillus* isolates were cultivated on nutrient agar at 37°C for 24 hours. The isolation depends on the shape of colonies and cells as well as on the biochemical tests.

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Calculation of Survival Rate

Bacterial suspension was prepared by transferring one colony of each culture into 15 mL tube containing 10 mL of nutrient broth. After gentil mixing, 200 µL of the Bacterial suspension was transferred to each well of a sterile 48 well plate. The control group contain untreated bacterial suspensions. Treatment groups contain bacterial suspensions that were treated with different laser parameters. The growth of control and treatment groups were measured using a microplate reader (BioTek 800 TS, USA) and the optical densities of all wells were recorded and denoted as OD at zero time, after the treatment (in case of treatment groups) and incubation for 24 hours, optical densities were recorded again and denoted as OD at 24 hours.

The bacterial growth was measured by subtracting the OD at zero time from the OD at 24 hours. The growth rate was measured by dividing the bacterial growth after treatment on the control growth.

Laser

Nd: YAG frequency-doubled laser (NIDEK SL 1600, Japan) emits green laser light (λ=532 nm) in the CW and chopped mode.

RESULTS

Effects of 532 nm Laser Light on the Viability of *Bacillus* spp.

Bacillus spp. irradiated with 532 nm laser light at an energy density of 77.8 J/cm^2 showed variable sensitivity. *B. licheniformis* was more sensitive than other strains, Figure 1.

Effect of Chopping Frequency on Bacterial Survival

The effect of chopping frequency was evaluated by using different frequencies 1.22, 1.61, 2.28 and 4.54 Hz with a constant exposure time (1-minute), output power (1 W) and energy density (77.8 J/cm$^{2)}$. *B.subtilis* was chosen to evaluate each laser parameter since it was the most resistant species. The survival rate of the bacteria was reduced with increasing chopping frequency as shown in Figure 2.

Effect of Exposure Times on Bacterial Survival

Figure 3 illustrates the survival rate of *B.subtilis*, irradiated with (532 nm) laser light at different exposure times and

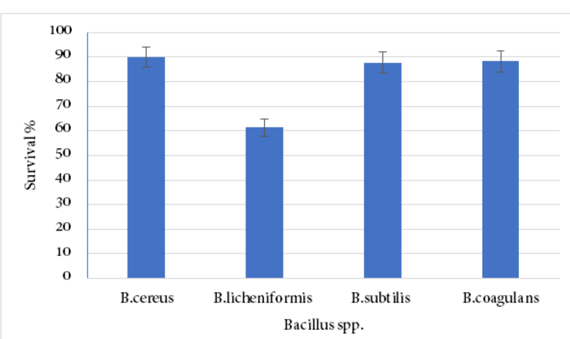

Figure 1: Sensitivity of *Bacillus spp.* to 532 nm laser light (time 1 min, chopping frequency 4.54 Hz, energy density = 77.8 J/cm^2).

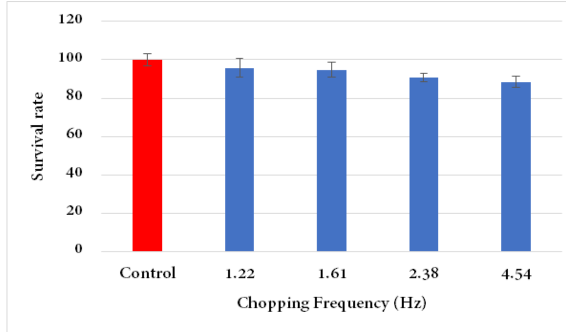

Figure 2: Effect of different chopping frequencies on the survival rate of *B. subtilis* strain. (exposure time = 1 min., energy density = 77.8 J/cm^2).

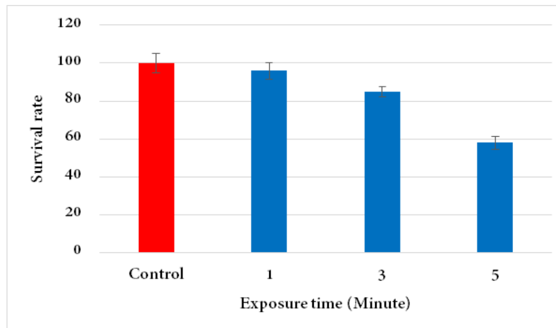

Figure 3: Irradiation of *B. subtilis* with (532 nm) laser light (1W) at different exposure times.

constant output power (1 W). The maximum effect of laser radiation appeared after irradiation time of 3 minutes.

Discussion

Several studies have investigated laser light's effect on bacterial growth and survival. According to the absorption spectra of a bacterial cell, it was found that the membranous respiratory chain components is the primary photo-acceptors of the visible and near infra-red light.[9]

Visible laser light at high intensities is known to have bactericidal effects mainly through reactive oxygen species (ROS) generation after light absorption by endogenous photosensitizers. The amount of ROS generated depends mainly on the light intensity, photosensitizer concentration and antioxidants produced by each bacterial strain.[7] This may partially explain the variation of radiation sensitivity among species of the same bacterial genus. Elevation of ROS is lethal to the cell, this effect is exploited in photodynamic therapy, which is employed in medicine for cancer therapy and antibacterial treatment.[10] while low-power white light enhances bacterial proliferation. The phototoxic effect was found to involve induction of ROS.

In addition to its effect via ROS generation, a fraction of the excited energy is transformed into heat during light excitation of electronic states, which causes a local rise in the temperature of the cellular molecules. This inurn may cause structural variations and/or activate biochemical reactions (second dark reactions) such as activation or inhibition of enzymes. This raise in local temperature can occur both in CW and pulse modes. The extant of this local temperature depends mainly on the intensity of the light.[10] while low-power white light enhances bacterial proliferation. The phototoxic effect was found to involve induction of ROS The effect of chopping frequency may attribute to the fact that increasing the irradiation frequency will decrease the relaxation time, this will increase the accumulation of both photochemical (ROS) and photothermal (heat) effects.[11] Single-cell irradiation studies using *Bacillus subtilis* as a gram-positive bacteria and members of gram-negative bacteria showed that gram-positive bacteria were more susceptible than gram-negative bacteria to irradiation-induced damage. The effect of 532 nm laser light is seen to be thermal at higher intensities and can even lead to cell destruction.[12] Photodynamic effects using appropriate photosensitizers could strongly enhance laser bactericidal activity.[13]

CONCLUSION

Laser energy deposited inside the cells increases with increasing repletion rate and irradiation time. This will increase the lethal effects of green laser by triggering intracellular mechanisms that interfere with the normal metabolic pathways leading to growth arrest.

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