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Research Article

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Effect of Electromagnetic radiation on *Lactobacillus* species

Sharsti Vasistha and Akshay Garg*

Department of Biotechnology, School of Biotechnology, Lovely Professional University, Jalandhar, Punjab, India

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ABSTRACT

Electromagnetic waves are used in today lives and spreading the environmental factor. We need visible light to see, radio waves for telecommunication and x-ray for medical purpose. Electromagnetic radiations are used for its bactericidal effect. It affects the cellular mechanism, cell membrane and might increase or decrease the activity of protein. The species selected for study was Lactobacillus rahmnosus (MTCC 1423) and Lactobacillus plantarum (MTCC 9496). Lactobacillus is friendly type of bacteria that lives in our digestive system, urinary tract and used to treat many skin disorders as blisters, canker sores, acne. The culture were exposed to three different frequencies (7.50GHz, 7.62GHz, 6.41GHz) with four different time intervals 5mins, 10 min, 15min, 20min. For the growth test MRS agar used and serial dilution were made to 10^{-3} and 10^{-4}. The result showed the significant decrease in the number of colonies (cfu/m) with increasing time and frequency, where as the antibiotic sensitivity test, antibiotics were Kanamycin (K30), Gentamicin (G50), Tetracycline (Te30), Norfloxacin (NX10) among all four antibiotics Norfloxacin (NX10) showed the maximum zone of inhibition and rest all have shown the random changes may be because of the activity of protein. The result of biochemical showed negative effect of electromagnetic radiation on Lactobacillus species.

Keywords: Electromagnetic radiation, *Lactobacilllus* species, Growth Test, Antibiotic Test.

INTRODUCTION

Lactic acid producing bacteria are gram positive, rod shaped and defined as the group of micro-aerophilic that ferments hexose sugars to produce primarily lactic acid [1]. It is used as a probiotic and beneficial to treat health related problem. Many genera of this bacteria spread widely in nature and have different effects in nature some are harmful or beneficial [2]. *Lactobacillus plantarum* and *Lactobacillus rhamnosus* found to have a antioxidant activity also helps to maintain intestinal Permeability [3]. *Lactobacillus* as a probiotic it could stop allergic reaction to rhinitis and used in medical research [4].

With the development of technology in waves, radiation has affected the organism as well as human beings. Electromagnetic radiation is a form of energy travels with the speed of light in space. The quantum radiation as a stream of energy called photons, each photon energy is considered to depend on the radiation frequency [5]. Cytogenic changes were studied in seeds when exposed to electromagnetic radiation (microwave) leads to the changes in chromosomal appearance, micro nuclei [6]. Exposure of electromagnetic radiation to bacteria proved to reduce the viability of cells after the treatment and the growth of bacteria also inhibited [7]. It was observed that height of plants decreases when exposed to radiation [10].

EXPERIMENTAL SECTION

Electromagnetic radiation device
Klystron power supply was used, device with low power and low voltage. It generates required beam and reflector voltage for X band. It is very stable and contains short circuit protection circuit. The beam voltage (300), beam current (0-40) and reflector voltage (70-75) were taken as standard.

Antimicrobial agents
Kanamycin(30), Gentamicin(50) , Tetracycline(30) and Norfloxacin(10) were obtained from Sigma Chemical.

Revival of bacteria
The ampoules of *Lactobacillus rhamnosus* (MTCC 1423) and *Lactobacillus plantarum* (MTCC 9496) were provided from Imtech Chandigarh and culturing is done in lovely professional university. The ampoules transferred into nutrient broth in two different flasks after that incubation is done for 48hrs. Then they were separated into two following culture:

i) Stock
ii) Working culture

Sub culturing
After 48hrs sub culturing of *Lactobacillus rhamnosus* and *Lactobacillus plantarum* was done in nutrient broth (100ml) separately in two different conical flasks.

Assessment the effect of Electromagnetic radiation
Growth Test
Pour MRS agar on the Petri plates. Cultures were exposed in two different frequencies. Serial dilution were made of 10^{-3} and 10^{-4}. Spreading was done with these dilutions with the use of ear buds. Incubate the plates for 24hrs.Count the colonies formed.

Antibiotic sensitivity Test
Pour the Muller Hinton on the Petri plates. The bacterial cultures were exposed to different frequencies. Spreading of cultures was done on the Petri plates with the use of ear buds. The antibiotic discs were fixed on Petri plates at different places. Incubation was done for 24hrs.Measure the zone of inhibition four different Antibiotics were Kanamycin(30), Gentamicin(50) , Tetracycline(30) and Norfloxacin(10).

Biochemical Test
MR – VP Test
10ml of MR-VP broth was taken in each test tube. In other test tube 5 ml of bacterial culture were taken and exposure was given. Then, a loopful containing exposed bacterial culture is inoculated in the broth. The tube is then incubated at 28ºC for 48 hrs. After incubation, a few drops of methyl red were added. Observe the changes in the color.

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Catalase Test
Taken a slide, on the slide added a drop of a culture. Then add a drop of 3% hydrogen peroxide. Seen bubble formed or not.

RESULTS AND DISCUSSION

Culture was collected after the revival of bacteria. Growth test showed the number of cells decreased when exposed to radiation with increasing frequency and time interval as compared to control (no exposure to radiation) for both the bacteria. At 10^{-3} more number of cells was observed than $10^{-4.}$

It was found that *Enterococcus hirae* (ATCC 9790) provided anaerobic condition and exposed to one hour electromagnetic radiation it leads to the decrease in the specific growth rate [11]. The study of Atli [12] showed that offspring of Drosophilla melangaster were reduced when exposed to microwave frequency radiation. With the study of Nafisi [2] the number of cells of *Escherichia coli* seems to decrease when exposed to low frequency radiation. When low frequency radiation provided to *Escherichia coli*, *Leclercia adecarboxylata* and *Staphylococcus aureus* it was found that number of colonies decreased [17].

The decrease in number of colonies could be due to, the radiation affects the cell membrane properties associated with ATPase activity [13]. It might have increased the oxidative effect on cell, and also the free radicals produce by electromagnetic radiation effected the growth of cells [14].

Also electromagnetic radiation causes damage to bacterial DNA and inhibition of its replication. [15] [16].

For antibiotics sensitivity test we observed in *Lactobacillus rhamnosus* Norfloxacin (NX50) having maximum zone of inhibition of 3.5cm without any radiation exposure (control). Its zone of inhibition of decreased randomly after exposure at different time interval. While the kanamycin showed the minimum zone of inhibition for all three frequencies. At frequency 6.41 GHz, the antibacterial activity of Gentamicin(G50) increased after radiation of 5mins, while its activity decreased at increased time of exposure. Kanamycin(k30) did not show its effect against *Lactobacillus plantarum.* At frequency 7.50 GHz and 7.62 GHz respectively, Kanamycin(k30) did not show any effect while Gentamicin(G50) showed maximum zone of inhibition at 5mins exposure of radiation and than decreased with increased time exposure.

The random results were seen in case of both *Lactobacillus plantarum* and *Lactobacillus rhamnosus* it may be because of protein activity. Electromagnetic radiation effect the cellular mechanism of bacteria [7]. It may be it affected the DNA [8] [9].

It might be the exposure time given to culture was not sufficient. At high frequency the significant zone of inhibition can be seen. It might be due to electromagnetic radiation has affected the cells shape. Biochemical tests showed the negative effects of electromagnetic radiation on *Lactobacillus species.*

CONCLUSION

Electromagnetic radiation affects the growth rate of bacteria, produce free radicals due to which the growth decreases and sometime it also inhibit the growth. Cell permeability also affected by the radiation. Low frequency might not affect zone of inhibition. From the data it can be easily decuced that the cellular membrane of microorganism affects by the electromagnetic radiation. Since the effect of EMR depends on the type of microorganism, intensity and the exposure duration. It also has further application like:

1. If any bacteria are having adverse effect we can use this radiation which may cause minimal damage to the cell but destroy other bacteria.
2. It may also help in the case of contamination of media.
3. Considering the potential of electromagnetic radiation application in controlling the growth of microorganisms and also implementation of these methods on new therapeutic protocols to control infections.
4. Formation of bacterial biofilm, and recovery from chronic, acute infection of wound, implant and bone infection.
5. This study shows that EMR induces a decrease in growth rate and morphological changes for gram-positive bacteria.

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