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Cellular effects of microwave radiations on *Escherichia coli*

Harmeet Kaur Uppal[1]*, Soma Chakki[2], Monika[1], Sujoy Dey[3]

[1]Department of Biotechnology, Mata Gujri College, Fatehgarh Sahib, Punjab-143602, (INDIA)

[2]Department of Genetics, Acharya Institute of Sciences, Bangalore, (INDIA)

[3]Department of Biotechnology, Ramaiah College of Arts, Science and Commerce, Bangalore, (INDIA)

Tel : +91-9463940859

E-mail : iamharmeet2002@yahoo.co.in

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ABSTRACT

This study was conducted in an attempt to characterize some of the effects of sublethal microwave radiation on cells of Escherichia coli O157:H7. Cultures were exposed to microwave radiation generated by microwave oven for 40, 80 and 120 seconds under controlled temperature conditions. The culture was then tested for cell viability, dormancy and cell wall leakage. Maximum destruction of *E.coli* cells was obtained at 120 sec. Experimental data shows that microwaves produced lethal effects on the examined bacteria in a manner that could not be explained solely by the thermal effects.

KEYWORDS

Microwave radiations;
Athermal effects;
Escherichia coli;
Electromagnetic radiations.

INTRODUCTION

Microwaves are a form of electromagnetic energy, like light waves or radio waves, and occupy a part of the electromagnetic spectrum of power, or energy. In our modern technological age, microwaves are used to relay long distance telephone signals, television programs, and computer information across the earth or to a satellite in space. But the microwave is most familiar to us as an energy source for cooking food[1]. In recent years, the use of microwave radiation has become popular in the food industry for thawing, drying, and baking foods, as well as for the inactivation of microorganisms in foods[2,3,4]. In particular, microbial destruction by microwave radiation has great potential in the pasteurization of foods. Its short heating and exposure time is less destructive to food than longer conventional heating. There have been several publications in recent years which have mentioned possible lethal effect of microwave on microbial species especially bacteria[5,6,7,8,9,10,11,12,11,12,13].

But there has always been controversy about the possible thermal and non thermal effects of microwave radiations. The thermal effects of microwave radiations have been proved by various scientists[14,15,16]. While there are many scientists who claim for the possible non-thermal effects of microwave processing[8,17,18,19].

The purpose of the present study was to introduce new methodology to ascertain the "athermic" (non- thermal) effects of microwave radiations emitted from microwave ovens on cells of *Escherichia coli*.

MATERIALS AND METHODS

Growth of bacterial culture

E.coli O157:H7 was obtained from Microbial Type

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Culture Collection and Gene Bank, Institute Of Microbial Technology, Chandigarh. The organism was maintained in LB broth (1% Bacto Tryptone, 0.5% yeast extract, 1% NaCl)[20]. The bacteria were cultured in 500ml of liquid medium at 37^{0}C for 24 h on a rotary shaker (150 rpm). 1ml of the culture was taken and was serially diluted in 0.9% NaCl solution. This dilution was divided equally into four different sterilized test tubes. Each one was marked as A, B, C, and D respectively.

Microwave treatment

For the microwave heating, a 2,450-MHz microwave oven (MS-1921 HE; LG Electronics) was used. Tube A was the control and was not exposed to Microwave radiation. Tube B was kept in a beaker containing ice and was treated with Microwave radiation for 40 seconds. Similarly tube C and tube D were treated for 80 and 120 seconds respectively in similar condition. Maximum temperature recorded after each exposure was 40^{0}C.

Measurements of viable cell counts and cell dormancy

After microwave treatment, 100 µl of solution was taken from each tube and spread onto LB Plates separately in LAF chamber in aseptic condition. The plates were incubated at 37^{0}C for 24 h, and cells were enumerated. To check the dormancy of cells, 100ml of inoculated LB media was taken and was treated with microwave radiations for about 5 seconds at 10 minute interval for 7 hours. Every half an hour, O.D was taken at 660 nm against a blank.

Detection of cell wall leakage

Another general indication of cellular damage to microorganisms is the leakage of nucleic acid and protein from cells. Microwave- injured cells have often been reported to release ninhydrin-positive material, purines, and pyrimidines into a suspension[17]. The presence of these materials in a suspension indicates damage to the cell at the membrane level. Furthermore, similarly injured cells are also known to release intracellular proteins into a suspension. The amount of protein released from the microwave-treated cells was measured at 600 nm by Lowry's method[21]. The DNA and RNA content of the supernatant was measured using DPA[22] and Orcinol method respectively. All the experiments were carried out in triplicate.

TABLE 1: Changes in the viable count of *E.coli* after microwave treatment

		Exposure time in Sec.			
		Control	40	80	120
Number of colonies	Plate: I	295	249	171	103
	Plate: II	288	236	165	92
	Plate: III	281	241	174	98
	Plate: IV	290	238	160	88
	Plate: V	295	245	158	108
	Average	290	242	165	98

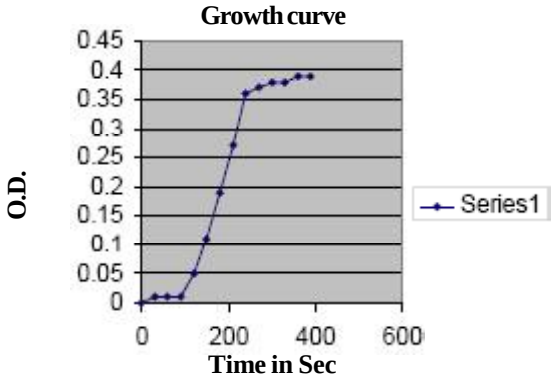

Figure 1 : Growth curve of *E.coli* cells not treated with microwave radiations

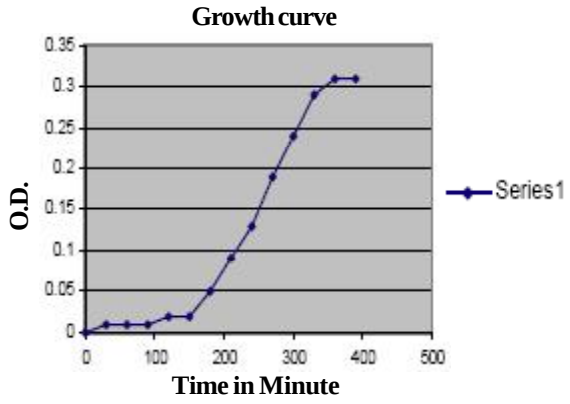

Figure 2 : Growth curve of *E.coli* cells exposed to microwave radiations

RESULT AND DISCUSSION

Inactivation of bacterial cells by microwave radiation

The inactivation patterns of the microwave-radiated cells were investigated by counting the number of cells in each of the four plates The viable counts were found to dramatically diminish relative to an increase in

TABLE 2: Estimation for the leakage of DNA from *E.coli* cells after microwave treatment

		Exposure time in Sec.			
		Control	40	80	120
O.D. at 600 nm	Tube: I	0.01	0.02	0.09	0.14
	Tube: II	0.01	0.03	0.09	0.13
	Tube: III	0.02	0.03	0.08	0.13
	Tube: IV	0.02	0.03	0.07	0.15
	Tube: V	0.01	0.04	0.08	0.13
	Average	0.014	0.03	0.08	0.13

TABLE 3: Estimation for the leakage of RNA from *E.coli* cells after microwave treatment

		Exposure time in Sec.			
		Control	40	80	120
O.D. at 600 nm	Tube: I	0.01	0.15	0.34	0.52
	Tube: II	0.01	0.16	0.39	0.53
	Tube: III	0.02	0.15	0.35	0.52
	Tube: IV	0.01	0.17	0.35	0.55
	Tube: V	0.02	0.17	0.34	0.55

TABLE 4: Estimation for the leakage of Proteins from *E.coli* cells after microwave treatment

		Exposure time in Sec.			
		Control	40	80	120
O.D. at 600 nm	Tube: I	0.01	0.15	0.34	0.52
	Tube: II	0.01	0.16	0.39	0.53
	Tube: III	0.02	0.15	0.35	0.52
	Tube: IV	0.01	0.17	0.35	0.55
	Tube: V	0.02	0.17	0.34	0.55
	Average	0.014	0.16	0.35	0.53

the microwave heating time(TABLE 1).

Upon microwave exposure some of the bacterial cells become dormant, thus the rate of multiplication decreases. But after some time they revive back and again start multiplying. Figure 1 shows the standard curve of *E.coli* cells which were not exposed to microwave radiations.

The growth curve of the bacterial cells after microwave treatment is shown in figure 2. Both the graph shows standard S-shaped growth curve. But due to microwave treatment the cells have undergone a state of dormancy and therefore graph II shows an extended lag phase whereas the untreated cells have a short lag phase followed by exponential phase.

Leakage of cell materials caused by microwave processing

Microwave radiation creates pores on the bacterial cell wall and it is through these pores that DNA, RNA and proteins are released into the solution. The amount of nucleic acid (DNA and RNA) released into the cell suspension was analyzed by DPA and Orcinol methods respectively. The absorbance was read at 600 nm. The amount of leaked nucleic acid from the cells grew relative to an increase in the time for which the cells were treated with microwave radiations. The results of leakage of DNA contents from the *E.coli* cells are shown in TABLE 2 and for the leakage of RNA contents in TABLE 3. The release of proteins into the suspension was also detected by Lowry's method and the absorbance was read at 600nm (TABLE 4).

CONCLUSION

The results of this investigation showed that even under contolled temperature conditions microwave radiations caused decreased viability and increased dormancy of *E.coli* cells. And this increased with the increase in the exposure time. Similarly there was leakage in the cellular contents of *E.coli*. These findings clearly indicate that apart from thermal effects microwave radiations also produce "athermal" effects which cause the killing of micro-organisms in ovens.

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