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Low intensity microwave radiation on *E. coli* host system for its compatibility and transformation efficiency

PRAVEEN KUMAR VEMURI*, SRI MANASWI TIPPARAJU, NISHANTH RAVI, MOHANA PRIYANKA RAMPATI, LAVANYA MUVVA, LAWANYA KONTHALA and V. V. MANOJ KUMAR BANDARU

*Department of Biotechnology, Koneru Lakshmaiah University, Vaddeswaram, Guntur District - 522 502 (India).

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ABSTRACT

Resonance-like biological effects of microwave radiation at frequencies of approximately 50 KHz on the growth rate of E. coli and on DNA have been reported in several scientific publications. In order to explore these non-thermal effects, we have measured the growth rate and the absorption spectrum of E. coli, irradiated by microwaves in the frequency range from 10 to 50 KHz. In addition, the effect of this radiation on plasmid DNA was studied by measuring plasmid transformation efficiency. Both the growth rate variations with varying frequency and the variations in the result of the plasmid transformation efficiency experiments were found to be statistically insignificant. The experimental results indicate that resonance effects are likely to have high transformation efficiency in this particular frequency range.

Key words: Microwave, transformation, *E. coli,* DNA and radiation.

INTRODUCTION

The use of microwave ovens in food industry is a growing trend. It is used for thawing, drying and cooking food, but the microorganism's inactivation that this treatment may exert or not is still a subject of worldwide discussion. For the past century microbiologists such as Duclaux[1], Henrici[2], and Hinshelwood[3] have shared the intuition that growth of bacterial cultures obeys certain rules that remained to be discovered. Monod[4] gave experimental support to that intuition by measuring the rate of growth and the yield of cells of cultures in media with crop-limiting concentrations of a single carbon source; both rate and yield depended upon the substance which served as the carbon source. Further support came from the seminal experiment of Schaechter et al.[5], who found a systematic change in size and composition of cells of *Salmonella typhimurium* when the growth rate varied by nutrition. Despite these and other important discoveries supporting the view that the rate of bacterial growth can be understood, that goal has not yet been attained. This study was carried out to investigate plasmid amplification, transformation efficiency and other cell diffe-rentiation by microwave irradiation in *E.coli.*

MATERIAL AND METHODS

Bacterial cultures and growth condi-tions

E.coli strain has been procured from National Collection of Industrial Microorganisms (NCIM), NCL-Pune and grown at 37°C in Luria Bertoni (LB) me-dium (10-g. tryptone, 5g. yeast extract and 10 g. NaCl for 1 liter). Bacterial growth was measured at 550 nm in spectrophotometer (ELICO SL-159, UV/VIS).

Amplification by Microwave Exposure

In these experi-ments, we used 10 KHz to 50 KHz microwaves in 55 W. (Imperial V- 8505T). The experiments carried out at 37°C (± 1 °C). Bacteria in logarithmic growth phase were exposed to microwave for 5 min every 15-min for 150 min. This irradiation procedure provided maximum irradiation effects.

Nucleic acid and protein determination

Protein content was determined by Lowry's method with BSA as standard[6]. Nucleic acid content was determined by optical density analytical measurement in UV spectrophotometer[7].

Plasmid puri-fication

Plasmid content in *E.coli* of 5-10 colonies was randomly selected and plasmid was prepared from 20ml culture. Plasmids from the bacteria were isolated by alkaline extraction method[8], where the lysozyme concentration was increased from 1 to 3 mg/ml and lysozyme was added to 100 units/ml. Plasmid DNA was purified by hydroxyapatite chromatography[9].

Amplification by Chloramphenicol

Bacteria were amplified with 170µg/ml of chloramphenicol that concluded by analogy to obtain the results of microwave irradiation.

RESULTS

1. It was identified that chloramphenicol inhibited the growth at a nearly 20%, Microwave inhibited the growth at a nearly 4% (Table 1, 2).
2. It was determined that microwave application decrease the protein amount at a rate of 6%, while chloramphenicol appli-cation did at a rate of 16% (Table 1, 2).
3. It was also found that the plasmid amount increased by 38% with chloramphenicol application; however, this increase was at a rate of 21% with microwave irradiation.
4. It was further found that the transformation efficiency with microwave treatment was observed maximum when exposed at 20 KHz for 5 minutes (Table 3).

Table 1: Data obtained from microwave exposure ex-periment

Minute	Endoprotein µg/ml	Growth A 550	Plasmid µg/ml
0.	0.950	1.300	0.330
15.	0.949	1.292	0.350
30.	0.930	1.287	0.360
45.	0.920	1.280	0.365
60.	0.910	1.279	0.367
75.	0.909	1.266	0.375
90.	0.900	1.266	0.378
105.	0.900	1.265	0.380
120.	0.899	1.259	0.384
135.	0.896	1.257	0.387
150.	0.897	1.254	0.390

Table 2: Data obtained from chloramphenicol treatment experiment

Minute	Endoprotein µg/ml	Growth A 550	Plasmid µg/ml
0	0.95	1.300	0.330
4	0.87	1.261	0.377
8	0.79	1.096	0.455
12	0.70	0.943	0.465

Table 3: Data obtained from Transformation efficiency experiment

Microwave intensity (for 5 minutes)	Concentration of plasmid DNA	Number of colonies	Transformation efficiency (%)
10 Hz	100ng/ul	1456	121
20 Hz	100ng/ul	1667	139
30 Hz	100ng/ul	492	41
40 Hz	100ng/ul	398	33
50 Hz	100ng/ul	255	28

DISCUSSION

As a result of the experiments carried out, the growth has been affected ad-versely from both microwave irradiation[10] and chloramphenicol application helps us think that they are effective, even diffe-rently, on division factors. The DNA amount decreases at the same rate as growth, although equal amounts of bacteria are taken in microwave irradiation; bring to mind that DNA is not replicated in cells[11, 1]. On the other hand in the chloramphenicol applica-tion, the decreasing of DNA amount with respect to the growth leads us to think that replication occurs, but that division does not. That protein amount is higher after microwave irradiation with respect to chloramphenicol indicates that microwave is so effective as chloramphenicol on protein synthesis mechanisms. To bring to mind that, since mi-crowave irradiation inhibits peptidyl transferase enzyme stability, chloramphenicol degrades portionally tertiary structure of one are more enzymes regarding the activity of protein synthesis system or that enzyme-substrate relation may have been degraded from ion unbal-ance or enzyme-substrate interaction[12]. Thus, it appears that the amplification formed by microwave irradiation does not constitute alternative when the time is not considered. However, when short time ir-radiation like 5 minutes is needed, it is an application that can constitute as an alter-native for amplification by antibiotics.

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REFERENCES

1. Duclaux, E. Traite de microbiologie. Masson, Paris. (1898-1901)
2. Henrici, A. T. Morphologic variation and rate of growth of bacteria. Microbiology monographs. Bailliere, Tindall, and Cox, London. (1928).
3. Hinshelwood, C. N. The chemical kinetics of the bacterial cell. Clarendon Press, Oxford. (1946).
4. Monod, J. Recherches sur la croissance des culture bacteriennes. Hermann et Cie, Paris. (1942).
5. Schaechter, M., O. Maal0e, and N. O. Kjeldgaard. Dependency on medium and temperature on cell size and chemical composition during balanced growth of Salmonella typhimurium. *J. Gen. Microbiol.* **19**: 592-606. (1958).
6. Bradford M.M. *Anal. Biochem.,* **72**: 248-254. (1976).
7. Davis L.G. In: Methods in Molecular Biology. Elsevier Science publishing. New York. (1986).
8. Birnboim H.C., Doly *J. Nucleic Acids Res.*, **7**: 1513 (1979).
9. Colman A., Byers M.J., Primrose S.B., Lyons A. Eur. J. Biochem., 91, 303-310. (1978).
10. Sagripanti J.L., Swicord M.L., Davis C.C. *Radiation Research*, **110**: 219-231. (1987)
11. Chipley J.R. *Advances in Appl. Micro-biol.,* **26**: 129-143 (1980)
12. Marka K., Musil J., Tuha H. In: Elec-tromagnetic Fields and The Life Environment. San Francisco Press INC. USA. (1971).