Microwave Influence in Fungi - a Preliminary Study

Al. Manoliu[a], Fl. M. Tufescu[b], L. Oprica[a], Z. Olteanu[a], D.E. Creanga[b*]

[a] Institute of Biological Research, 20 A Bd. Carol I, 6600 Iasi, Romania, e-mail: Dorina.Creanga@email.ro
[b]Univ. Al. I. Cuza, Fac. of Physics, 20 A Bd. Carol I, 6600 Iasi, Romania

Abstract. The behavior of two cellulolytic fungus species under the influence of low intensity microwaves was studied: *Chaetomium globosum* and *Alternaria alternata*. Enzyme activity of dehydrogenase complex was investigated by spectrophotometric method in order to reveal the effect of relatively short daily exposure times. Inhibitory effect was noticed for malate dehydrogenase and succinate dehydrogenase in both fungi while differentiated influence was revealed in alpha ceto glutarate dehydrogenase (inhibitory in *Chaetomium globosum* but stimulatory in *Alternaria alternata*). Isocitrate dehydrogenase activity was significantly stimulated in both fungi for 3 hours exposure time.

1. Introduction

Some literature reports mention the influence of the magnetic treatment on enzyme activity in some microorganisms, especially bacteria:

-various bacterial strains that have been exposed to a homogeneous magnetic field of 1 Tesla, presented no mutagenic or lethal effects, the activity of the bacterial enzyme beta-galactosidase being also found to be independent of the applied magnetic field [1];

-the stimulatory effect of inhomogeneous 2-6.1 T magnetic fields on beta-galactosidase activity in *E. coli* body cell was reported [2];

-experimental investigation on *E. coli* biochemical behavior after magnetic treatment showed that certain ranges of low frequency electromagnetic waves can influence the activity of a cytoplasmic enzyme, the enolase. Depending on the intensity of electromagnetic waves, the enolase level could be enhanced or diminished though no linear dependence was emphasized [3];

-experiments carried out on viruses revealed that microwaves are able to destroy DNA structure although a satisfactory explanation, based on energies and absorption phenomena, could not be developed; Kakita showed that the microwave effect is distinguishable from external heating by the fact that it is capable of extensively fragmenting viral DNA, something that heating to the same temperature did not accomplish [4];

-in bacteria exposed to microwaves of high power density, lethal effect was obtained [5], fact which was correlated with sterilization techniques;

-in beer yeast Saccharomyces cerevisiae [6-7] the growth rate diminution was noticed;

-in beer yeast Saccharomyces cerevisiae as well as in E. coli bacteria, continuous wave exposure did not lead to non-thermal effects [8];

-the lack of effects after exposure to microwaves of beer yeast cells was reported also in [9], when working 2.45 HGz waves;

-the enhance of DNA biosynthesis rate was obtained in the fungus Physarium polycephalum [10];

- researches carried out on the fungus Aspergillus amstelodami confirmed the lack of mutations after 8.7 GHz exposure [11].

In the preliminary study presented below the effect of microwaves (MW) on the cellulasic properties of *Chaetomium globosum* and *Alternaria alternata* is analyzed.

2. Material and method

Biological material, physical treatment, measurement method and statistical analysis are given in the next.

2.1. Biological material

Two cellulolytic fungus species were studied: *Chaetomium globosum* and *Alternaria alternata* (used in the cellulose biotechnology). The cultures intended for MW exposure were developed on agar-agar culture medium in Petri dishes of 9.5 cm diameter, each containing 20 ml of solid Haynes medium (12). MW exposure lasted for one week (six days), exposure times being equal to 1 hour, 3 hours and respectively 8 hours daily. Then fungi cultures were transferred on liquid medium and let to grow for 11 days at constant temperature - equal to 24 degrees Celsius (thermostat sterile room). After 7 days as well as at the end of the growth period (the 11th day), the dehydrogenase activity values were determined. The experiment was repeated five times in the same conditions.

2.2. Physical device

The microwave generator, based on an IMPATT diode, was provided with a horn antenna, being able to deliver a MW flow with a low density power (of 1 mW/cm^2), as measured with a probe magnetometer, at a frequency of 10.75 ±0.5 GHz.

2.3. Enzyme activity assay

Dehydrogenase assay in microorganism biomass was carried out according to Sisoev and Krasna method (modified by Artenie, [13]). Basically, this measured the capacity of hydrogen transfer from various substrates to 2,3,5-three phenyl tetrazolium, which is reduced, becoming three-phenyl formasan, colored in red. The intensity of this color (measured spectrophotometrically at the wavelength of 540 nm) is proportional to the enzyme activity. JASCO (Japan) spectrophotometer was used for samples and standard solutions assay. Enzyme activity is expressed as the amount (micrograms) of reduced three-phenyl tetrazolium for every gram of microbial biomass.

2.4. Statistical analysis

The changes of enzyme activity in every exposed sample in comparison to the control ones (changes induced by microwave exposure) were statistically analyzed by means of Student t-test, pair, two tailed. In Table 1 the values of ***p***-probabilistic parameter (the level of significance) are given.

3. Results and discussion

The results obtained for the fungus *Chaetomium globosum* are represented in Figures 1 a-d, where the enzyme activity after 7 days is marked with white bars while the situation after 11 days is marked with black bars. In figure 1 a is given the activity of alpha ceto glutarate dehydrogenase.

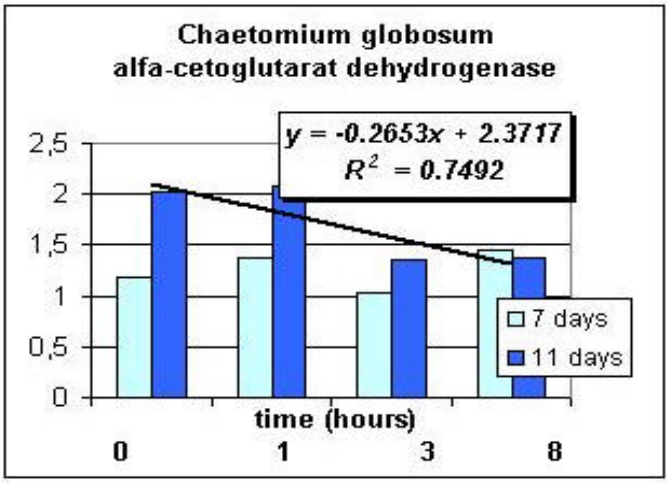

FIG. 1 a. *Microwave action in Chaetomium globosum fungus: the activity of alpha ceto glutarate dehydrogenase*

After 11 days the enzyme activity is significantly decreased for the exposure times of 3 and 8 hours, although after 7 days a slight enhancing tendency is suggested by the graph. However, after 7 days of growth, the level of significance (Table 1) is $p>0.05$ for exposed samples corresponding to 1 hour and 8 hours while for 3 hours we obtained $p<0.05$, meaning significant modifications for the enhanced enzyme activity. Since the level of significance for the exposed samples that present diminished activity after 11 days is $p<0.001$ we may conclude that this enzyme appears to have a lower activity after microwave exposure than in control culture. Linear negative dependence of enzyme activity on the exposure time was emphasized, the correlation coefficient being $R^2=0.6313$.

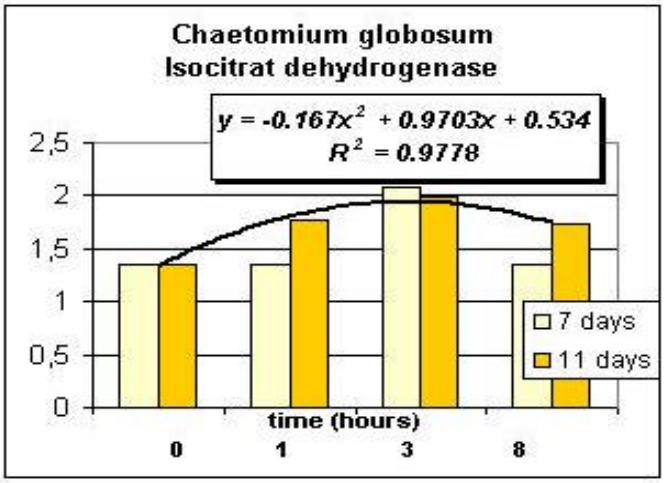

FIG. 1 b. *Microwave action in Chaetomium globosum fungus: the activity of isocitrate dehydrogenase*

The situation of isocitrate dehydrogenase activity is presented in Figure 1 b, where a remarkable parallelism between the values after 7 days and those after 11 days of growth can be seen. While in the control culture there is no difference between the 7th and the 11th day situations, in all exposed samples enzyme activity is higher after 11 days than after 7 days. Polynomial dependence of isocitrate dehydrogenase activity on daily exposure time was outlined, with a correlation coefficient equal to $R^2=0.9469$. Table 1 confirms the stimulator effect of microwaves, all sample values at 11 days being modified statistically significant in comparison to the control sample.

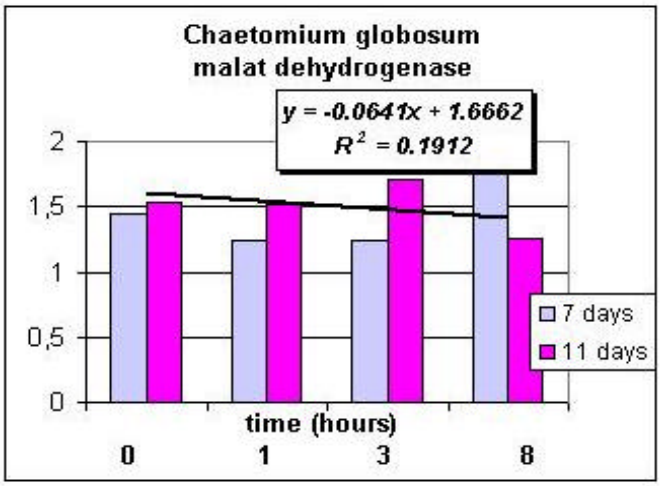

FIG. 1 c. *Microwave action in Chaetomium globosum fungus: the activity of malate dehydrogenase*

In Figure 1 c the malate dehydrogenase behavior is given. Neither after 7 days of fungus growth nor after 11 days of development was there a clear tendency of variation in exposed sample in comparison to the control sample. For the exposure time of 8 hours, on day 7, as well as for the exposure time of 3 hours on day 11, the malate dehydrogenase activity is non-significantly changed in comparison to the control - level of significance: $p>0.05$. This change suggested a slight diminution of

enzyme activity following the daily exposure to microwaves; linear dependence on the exposure time, obtained by computational processing, is characterized by low correlation coefficient of 0.4559.

Table I. Statistical analysis of spectral measurements (five repetitions) carried out by Student t-test

C. globosum 7 days	**Alpha cetoglutarate dehydrogenase**	**Iso citrate dehydrogenase**	**Malate dehydrogenase**	**Succinate dehydrogenase**
1 hour	P>0.05	p>0.05*	P<0.05*	P<0.05
3 hours	P<0.05	P<0.05	P<0.05*	P>0.05
8 hours	p>0.05*	p>0.05*	P>0.05	P>0.05
C. globosum 11 days				
1 hour	P<0.001	P<0.05	P<0.05*	P<0.05
3 hours	P<0.001	P<0.05	P>0.05*	P<0.05
8 hours	P<0.001	P<0.05	P<0.05*	P<0.05
A. alternata 7 days				
1 hour	P<0.05	P<0.05	p>0.05*	p>0.05*
3 hours	P<0.05	P<0.05	p>0.05*	p>0.05*
8 hours	p>0.05*	p>0.05*	p>0.05*	p>0.05*
A. alternata 11 days				
1 hour	P<0.05	P<0.05	p>0.05*	P<0.05*
3 hours	P<0.05	P<0.05	p>0.05*	P<0.05*
8 hours	P<0.05	P>0.05	p>0.05*	p>0.05*
* statistically non-significant				

Succinate dehydrogenase activity (figure 1 d) is clearly diminished (all exposed samples are characterized by statistically significant changes according to Table I) after 11 days of fungus growth under daily exposure to MW. Exponential function may approximate the decrease of succinate dehydrogenase activity to the increase of MW exposure time, with a correlation coefficient of only 0.3296. Non-significant enhancements after 3 hour and 8 hours of MW exposure were noticed at day 7.

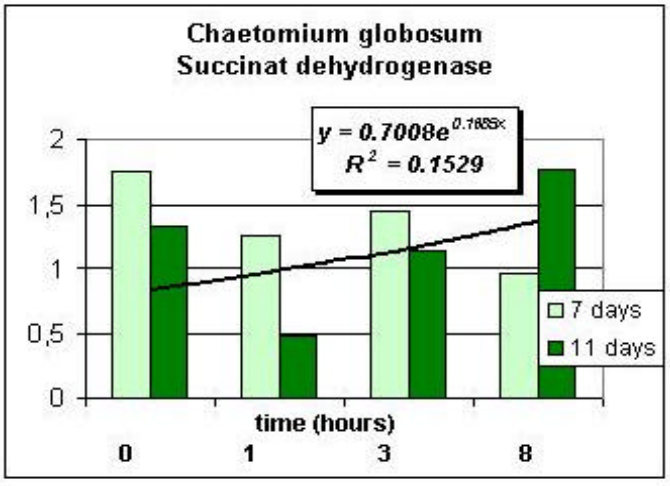

FIG. 1 d. *Microwave action in Chaetomium globosum fungus: the activity of succinate dehydrogenase*

In *Alternaria alternata*, the activity of alpha ceto glutarate dehydrogenase is clearly enhanced after microwave treatment, in contrast with the case of *Chaetomium globosum* (Figure 2a). The linear dependence of enzyme activity on the daily exposure time to microwaves is characterized by correlation coefficient equal to 0.7217.

If we look to the 7th day situation, we see that microwave exposure for 8 hours is able to induce a very small enhancement in the enzyme activity - significantly lower than the exposures for 1 hour and 3 hours (level of significance p>0.05 for 8 hours, but p<0.05 for 1 and 3 hours). This is

concordant with the situation observed on day 11 where all exposed samples are significantly enhanced in comparison to the control.

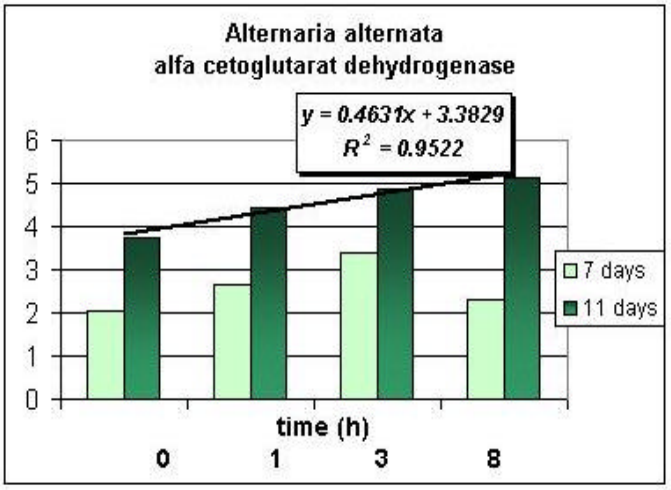

FIG .2.a. *Microwave action in Alternaria alternata fungus: the activity of alpha ceto glutarate dehydrogenase*

In Figure 2 b the behavior of isocitrate dehydrogenase activity is given. Variation similar to that noticed in *Chaetomium globosum* can be seen: polynomial variation of enzyme activity to the microwave exposure time increasing (correlation coefficient 0.821).

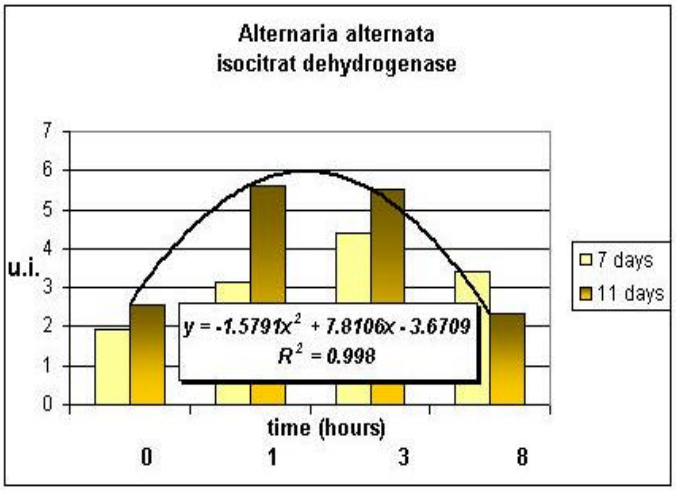

FIG .2.b. *Microwave action in Alternaria alternata fungus: the activity of isocitrate glutarate dehydrogenase*

Quantitatively, the microwave stimulator effect is much more important in *Alternaria alternata* than in *Chaetomium globosum,* since the enhancement of the sample corresponding to daily exposure time of 3 hours represents more than 100% while in the other fungus this enhancement was only 30%.
The highest exposure time led to an inhibitory effect as one can see from the value on day 11 which is lower than that of day 7 and it is also lower than that of the control sample (the difference being not statistically significant: $p>0.05$).The response of malate dehydrogenase (Figure 2 c) is rather analogous to that corresponding to the other fungus, no significant influence of microwave exposure being revealed. Relatively slight correlation coefficient of experimental data around the theoretical regression curve (0.6663) is concordant with the low statistical significance level of exposed samples in comparison to the control sample: $p>0.05$. In Figure 2 d we present the influence of daily microwave exposure upon the enzyme activity for succinate dehydrogenase. There seems to be an inhibitor effect described by a theoretical polynomial variation in exposed samples in comparison to the control ones on day 11 (correlation coefficient is 0.9894). However, on day 7 one can see some slight enhancement in the samples corresponding to low exposure times: 1 hour and 3 hours.

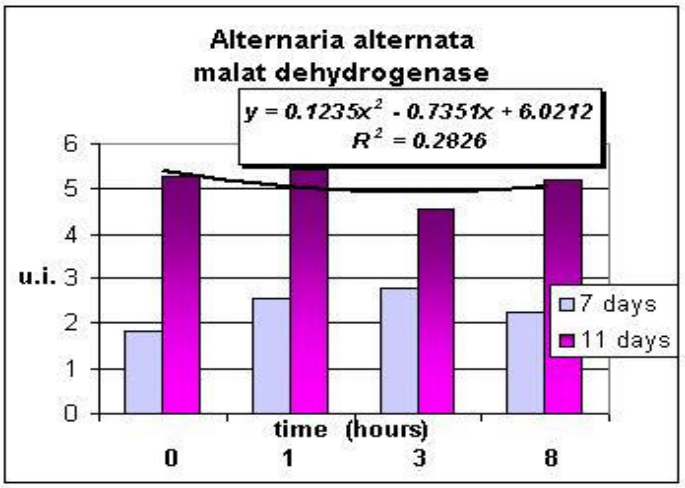

FIG .2.c. *Microwave action in Alternaria alternata fungus: the activity of malate dehydrogenase*

Their p-values also indicate non-significant modifications (Table 1). To conclude on the behavior of the two cellullolytic fungi species, treated with low power density MW flows, we may say that repeated exposures are able to influence enzyme activity in both fungi strains. Generally it is an inhibitory effect, except for alpha-ceto glutamate dehydrogenase that is stimulated in *Alternaria alternata* after 11 days of growth.

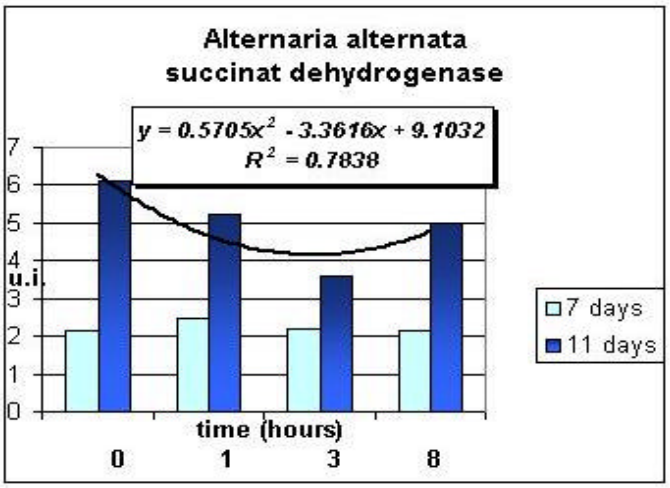

FIG .2.d. *Microwave action in Alternaria alternata fungus: the activity of succinate dehydrogenase*

In addition the isocitrate dehydrogenase is enhanced in both *Chaetomium globosum* and *Alternaria alternata* in the sample corresponding to 3 hours daily exposure to MW.

To explain such behavior one needs to accept that at least two main influences, with different effects on enzyme activity, are induced by microwaves in fungi cultures. MW may influence directly enzyme activity of proteic structures, by destabilizing their hydrogen bonds in the specific situs– responsible for enzyme character of proteins. Microwaves may also have more complex influence on the cell biosynthesis phenomena, this influence being a positive or a negative one, depending on power density level, microorganism species or physiological state. These two concurrent types of effects may result in enhance or diminution of final enzyme activity. Perhaps specific biosynthesis phenomena corresponding to isocitrate dehydrogenase are stimulated by microwave action being able to dominate damages induced by microwaves in hydrogen bonds system but further experiments are needed, based on deeper investigation methods, to clarify this finding.

To assign microwave effect in fungi enzymes to thermal or athermal effect is rather difficult. We choose to work with low power density (<1 $mWcm^{-2}$), so, thermal effect, quite low in the case of our experiment, is expected to cause a slight increase of temperature in the irradiated samples (due to

the dielectric relaxation of water molecules), being associated with a stimulation of enzyme biosynthesis. But the decrease of enzyme activity levels may be related to a non-thermal, specific, effect of microwaves in living tissues (coexisting with the thermal one) that dominates only in the case of some enzymes.

The inhibitory action deriving from the non-thermal microwave effect may consist in the perturbation of ion channels functions at the level of membrane structures of the cell. Due to the discovery of Na^+ ion channels voltage dependent in the bacteria membrane we may assume that in fungus membrane exist also such structures. The electro sensitivity of this proteic aggregates is assured by several amino acids residues with distinct electric charge that may be easier influenced by though ion channels of plasma membrane and other cytoplasmatic structures are also supposed to experience microwave action. Perturbation of ion transport may lead to perturbations of various biochemical reactions which are controlled by ion messengers; so it is possible that biochemical reactions involved in the enzyme biosynthesis are delayed for the duration of MW action.

Nevertheless, molecular effect of MW could occur at the level of protein structures, mainly since their enzyme activity is based on relatively weak forces (hydrogen bonds). Regarding chemical bonds assuring protein primary structure, it is not impossible that electromagnetic fields are able to destroy them as well, since recent studies demonstrated such destruction for nucleic acid chemical bonds when exposed to microwaves [4]. Microwave photon energy is millions of times lower than that of chemical or even hydrogen bonds so that the absorption of a microwave photon cannot break these bonds. But more complex, indirect, phenomena, possible within cell complex structures, may occur. May be the accumulation of oxygen radicals, is the primary cause, as they are suspected for dissociating the covalent bonds of DNA [14], [15] when water molecules are adsorbed on the surface of large biomolecules within cellular medium. But, we might say, this is also the case of proteic structures, particularly enzyme molecules. Oxygen radicals can be generated by the disruption of a hydrogen bond on a water molecule. Water molecules distributed alongside enzyme molecules as "bound" water, are characterized by complicated dynamics since they interact both with protein atoms and with surrounding medium molecules, so, eventually, hydrogen atom liberation could occur, especially when a certain thermal effect of microwaves is involved.

4. Conclusion

Preliminary test on microwave effect in fungi enzyme showed a general negative influence, both in *Chaetomium globosum* and *Alternaria alternata*. Exceptional increase of enzyme activity of alpha cetoglutarate dehydrogenase in *Alternaria alternata* as well as the increase of isocitrate dehydrogenase activity for relatively short exposure times (1 and 3 hours) in both fungus species, were noticed. The amplitude of microwave stimulator effect upon isocitrate dehydrogenase corresponding to 3 hours exposure time is 3 times higher in *Alternaria alternata* than in *Chaetomium globosum.* Since power density level was rather low, non-thermal effect is supposed to dominate in both tested fungi. Complex concurrent phenomena may occur under microwave action: the damage of enzyme structure and function as well as enzyme biosynthesis stimulation or inhibition: no information upon the sense of microwave action in enzyme biosynthesis could be obtained during our spectral investigation. Further tests are to be designed in order to enlarge image concerning molecular mechanisms acting in fungus cell especially with the aim of design new biotechnological tools based on low intensity microwaves.

REFERENCES

1. Thomas, A., Morris, P.G. *The Effects of NMR Exposure on Living Organisms*. I. A Microbial Assay. Br. J. Radiol. *54* (643): 615-621,(1981)
2. Tsuchiya, K., Okuno, K., Ano, T., Tanaka, K., Takahashi, H., Shoda, M. *High Magnetic Field Enhances Stationary Phase-Specific Transcription Activity of Escherichia Coli*. Bioelectrochem. Bioenerg., *48* (2): 383-387,(1999)
3. Dutta, S.K., Verma, M., Blackman. *C.F. Frequency-Dependent Alterations in Enolase Activity in Escherichia Coli Caused by Exposure to Electric and Magnetic Fields*. Bioelectromagnetics, *15* (5): 377-383 (1994)

4. Kakita, Y., N. Kashige, K. Murata, A. Kuroiwa, M. Funatsu, Watanabe, K., *Inactivation of Lactobacillus bacteriophage PL-1 by microwave irradiation.* Microbiol. Immunol. 39: 571-576, (1995)
5. Daradlhon, M., Averbeck, D., in *Proceedings of 9ieme Congres International de la Societe Francaise de Radioprotection, 1978*, Nainville-les-Roches, 279
6. Thourel, L., et al., in *Proceedings of IMPI Symp*., 1975, Waterloo, Canada, 127,
7. Grundler, W., et al., *Non thermal resonant effects of 42 GHz microwaves on the growth of yeast cultures*, Heidelberg, Springer Verlag, 21, (1983)
8. Blackman, C., et al., in *Proceedings of Ann. New York Acad. Sci*, 1975, 247, 352
9. Dutta, S., et al., *Lack of microbial genetic response to 2.45 GHz CW and 8.5 to 9.6 GHz pulsed microwaves*. J. Cell. Biol., 63 (2), 90, (1974)
10. Mezykovsky, T., et al., *Response of Aspergilus nidulans and Physarium polycephalum to microwave irradiation*. J. Microw. Power, 15(2): 75 ,(1980)
11. Dhahi, S., et al., *Lack of mutagenic effects on conidia of Aspergillus amstelodami irradiated by 8.7175 GHz CW microwaves.* J. Microw. Power, 17 (4): 345, (1982)
12. Zarnea, G., *Tratat de microbiologie generala,* Ed. Acad. Rom., Bucuresti, (1982)
13. Artenie, V., *Practicum de biochimie*. Ed. Univ. Al. I. Cuza, Iasi, (1987)
14. Kashige, N., Kojima, M., Watanabe, K., *Correlation between DNA-breaking activity of aminosugars and the amounts of active oxygen molecules generated in their aqueous solutions*. Agric. Biol. Chem. 55: 1497-1505, (1990)
15. Kashige, N., Yamaguchi, T., Ohtakara, A., Mitsutomi, M., Brimacombe, J. S. Miake, F., Watanabe, K. *Structure-activity relationships in the induction of single-strand breakage in plasmid pBR322 DNA by amino sugars and derivatives.* Carbohydrate Research 257, 285-291, (1994)