Biological Effect of Non-Ionizing Radiations on Microorganisms

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ABSTRACT

We studied the effect of extremely low frequency magnetic fields (ELF-MF) of 60-Hz and 500 mT on the growth and the mutation frequency of the budding yeast *S.cerevisiae* and on the behavior of the ciliate *Paramecium multimicronucleatum*. The growth rate and mutation frequencies of several strains of S.cerevisiae (wild type and radiation sensitive mutants, rad or rev) were examined but no significant difference was observed. Moreover, the behavior of *P.multimicronucleatum* under the ELF-MF was examined. When exposed to a vertical filed of 0.6 T, the cells accumulated at the upper end of the cuvette.

INTRODUCTION

The question of whether ELF-MF exerts biological effects such as growth inhibition or cancer risk is confounded to the date by both positive and negative reports. Epidemiological studies have shown correlations between ELF-MF exposure and the incidence of cancers (1). Moreover basic biological events such as cell cycle (2) and DNA replication (3) were affected by exposure of ELF- MF. However, negative reports had been accumulated.

In this report, we tried to elucidate the ELF-MF effect at the molecular level, and as the model organisms of this problem we used the yeast *S.cerevisia*e, and the ciliate *Paramecium multimicronucleatum*.

MATERIALS AND METHODS

Strains

The following strains were used:

Yeast: *S.cerevisiae*, S288C (*MATα mal gal2*), X12-6B (*rad1*), X16-9C *(rad2)*, X36B-3C *(rad3)*, X10-1C (*rad6*), X56-10A (*rad9*), JG-18 (*rad18*), XS133-3B (*rad51*), g160/2b (*rad52*), X1687-101b (*rad55*), 16C-235 (*rev1*), 16C-63 (*rev2*),and 16C-184 (*rev3*)

Paramecium: *P.multimicronucleatum*, stock CH

Magnetic fields exposure system

Magnetic fields were generated with 2 pairs of coils (100 x 100 mm) 20 mm apart. The power source is AC 200V, 60Hz. The generated field has a magnetic intensity from 0 to 500 mT. Static magnetic field was generated with 2 pairs of magnets (ϕ50 mm) 15 mm apart. The magnetic intensity is 500 mT.

Measurement of cell growth

The yeasts and ciliates were exposed with magnetic fields on the petri dish (ϕ100 mm or ϕ50 mm) during growth, and the lengths of colonies and number of cells were measured respectively.

Measurement of mutation frequency

The reversion rates of *ade2-1* were measured using the Fluctuation test (4).

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Observing the the behavior of P.multimicronucleatum

The system for observing the behavior of *P.multimicronucleatum* under the FLF magnetic field is illustrated in Fig.1. *P.multimicronucleatum* was cultured in a hay infusion inoculated with *Klebsiella pneumoniae*. Paramecium cells at early stationary phase were collected by low-speed centrifugation and suspended in a solution containing 1 mm CaCl2, 0.5 mM ,1mM KCl and 2mM Tris-HCl (pH7.2). The cells were transferred into an observation cuvette made of polystyrene (46 x 10 x 10 mm^3), and left undisturbed for at least 30 min before starting the experiments. For the temperature control, the water flow from a bath cooler was indirectly circulated around the coil. The temperature around the cuvette was kept at 22 ± 0.5 °C. The video images were displayed on a monitor (PVM-1454Q, Sony, Japan) and a camera placed in front of the screen photographed cell distribution or swimming track with 1/8 or 2 s exposure (5).

RESULTS AND DISCUSSION

Exposure of static and extremely low frequency magnetic fields to yeast did not affect both cell growth and killing (table 1 and 2). We used wild type and radiation sensitive mutants (*rad* or *rev*) yeasts (6). The growth of all strains tested did not change with exposure of static MF or ELF-MF. The growth of ciliates was not affected by the exposure of the magnetic fields (data not shown).

Table 1. Average doubling time of the yeast

	static MF		ELF-MF	control
time (min)	86± 4	87 ± 5	90 ± 5	

ELF-MF exposure did not increase the reversion frequency of *S.cerevisiae ade2-1* gene (table 3.).

Recently 50-Hz ELF-MF induced mutations in the hypoxanthin-guanine phosphoribosyl transferase gene of human melanoma cells, and DNA replication error is suspected of causing the mutation by ELF-MF exposure. ELF-MF is not considered to cause DNA damage and strand breaks because ELF-MF has too very weak energy to attack DNA directly (7, 8, 9). Moreover, radiation sensitive mutations (*rad* or *rev*) had no effect on ELF-MF exposure to cells. This means that DNA repair system of the yeast has no relation to ELF-MF effect.

Table 2. Average length of colonies.

strain	ELF-MF [colony length (ϕ mm)]	static MF	control
S288c (RAD+)	2.8 ± 0.3	3.3 ± 0.2	2.9 ± 0.2
X12-6B (*rad1*)	2.8 ± 0.2	3.1 ± 0.2	3.0 ± 0.3
X16-9C *(rad2)*	2.4 ± 0.3	2.6 ± 0.2	2.3 ± 0.2
X36B-3C *(rad3)*	2.4 ± 0.2	2.6 ± 0.2	2.5 ± 0.2
X10-1C (*rad6*)	2.6 ± 0.2	2.7 ± 0.3	2.6 ± 0.2
X56-10A (*rad9*)	2.7 ± 0.2	2.6 ± 0.3	2.7 ± 0.3
JG-18 (*rad18*)	2.7 ± 0.2	2.5 ± 0.2	2.4 ± 0.2
XS133-3B (*rad51*)	2.8 ± 0.3	3.0 ± 0.4	2.6 ± 0.3
g160/2b (*rad52*)	2.3 ± 0.2	2.2 ± 0.2	2.3 ± 0.2
X1687-101b (*rad55*)	2.7 ± 0.2	2.6 ± 0.2	2.9 ± 0.3
16C-235 (*rev1*)	2.4 ± 0.2	2.5 ± 0.2	2.3 ± 0.2
16C-63 (*rev2*)	2.3 ± 0.2	2.2 ± 0.2	2.1 ± 0.2
16C-184 (*rev3*)	2.7 ± 0.3	2.9 ± 0.3	2.6 ± 0.3

Table 3. Reversion frequency of *ade2-1* gene

strain	ELF [rev. freq. ($\times 10^{-8}$)]	control
W303-1a (RAD^+REV^+)	3.1 ± 0.4	3.2 ± 0.5
X12-6B (*rad1*)	4.5 ± 0.6	4.2 ± 0.7
X59-10A (*rad9*)	5.2 ± 0.7	5.5 ± 0.8
JG-18 (*rad18*)	3.4 ± 0.4	3.4 ± 0.4
16C-184 (*rev3*)	1.2 ± 0.1	1.4 ± 0.2

The behavior of the ciliate *P.multimicronucleatum* was observed by the CCD camera system (see Figure 1). A control observation without application of ELF-MF over 30 min showed a slight accumulation upside of the cuvette; the percentage of cells that accumulated in the upper half was 55 to 60% of the total within observation window. Exposure to the FLE-MF altered the vertical distribution of the cells. At 3 min following 0.59 T exposure, the cells remarkably accumulated upside of the cuvette. When the intensity of the magnetic field was changed, accumulation in the upper half was induced at about 0.4 T (Figure 2). This result implies that the ELF-MF enhances the negative gravitaxis. Although how the magnetic field induces the gradual changes in swimming orientation is not determined yet, it seems that the accumulation upside is based on the enhancement of sensitivity to gravity. More detailed observations of the *Paramecium* cell in magnetic fields will reveal the action of magnetic field on the cell.

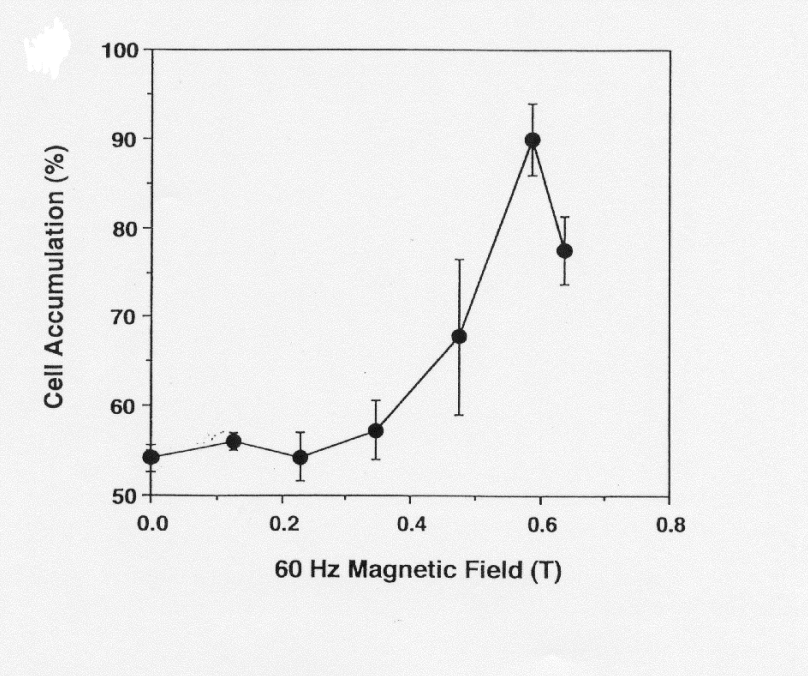

Figure 2. Vertical distribution of *Paramecium*

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