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Short communication

Static and 50 Hz magnetic fields of 0.35 and 2.45 mT have no effect on the growth of *Saccharomyces cerevisiae*

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Abstract

The present work reports the growth effects induced by static and sinusoidal 50 Hz magnetic fields (MF) on the haploid yeast strain *Saccharomyces cerevisiae* WS8105-1C. Magnetic fields were generated by a pair of Helmholtz coils (40 cm in diameter) with 154 turns of copper wire in each and separated 20 cm. The experiments were performed at 0.35 and 2.45 mT, and yeasts were exposed to MF during 24 and 72 h in the homogeneous field area. Growth was monitored by measuring the optical density at 600 nm. The data presented in the current report indicate that static and sinusoidal 50 Hz MF (0.35 and 2.45 mT) do not induce alterations in the growth of *S. cerevisiae*.
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1. Introduction

The increasing exposure of the population to electromagnetic fields (EMF) in everyday life has enhanced public interest in epidemiological and experimental studies. It has been published that job-related, environmental, and residential exposures to EMF are implicated in the development of human cancers [1,2]; nevertheless, other epidemiological studies have not found this correlation [3]. Under laboratory conditions, a wide variety of effects has been published; nevertheless, some other related experimental studies have not confirmed these previous findings—resulting discordant probably by the use of different EMF exposure protocols, frequencies, intensities, waveforms, and cell types [4–9]. Therefore, the scientific debate established is still nowadays open.

The budding yeast (*Saccharomyces cerevisiae*) has become an excellent eukaryotic model to explore the EMF effects on cell growth due to the well-characterized morphological, metabolic, and genetic characteristics and their conserved similarity in molecular mechanisms associated with basic cellular processes, among different eukaryotic species including human cells [10].

Studies concerning the use of *S. cerevisiae* as a cell model have reported that proliferation increases more than 25% at 50 Hz, 0.5 mT alternating magnetic fields (MF) during 10-h exposure [11]. Nevertheless, the effect found by these authors when they used 0.2 mT, 50 Hz, in the exposition is the inhibitory action (16%) of cell proliferation. The cell growth dependence with field frequency found by them shows positive responses by more than 20% increase in the cell number after 10 h of exposure to 15 and 50 Hz. These authors suggest the existence of frequency 'windows' of proliferation. In relation to studies that have used higher MF intensity, Motta et al. [12] have found an increment in the proliferation of yeast cultures exposed to 110 and 220 mT steady MF.

In contrast to these observations, Nakasono et al. [13] have not found changes in the expression of proteins or genes related to heat-shock response, DNA repair, respi-

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ration, protein synthesis, and cell cycle after exposure of yeast to 50 Hz and 10, 150, or 300 mT for 24 h. In the same way, when stress-responsive proteins were analyzed in cells exposed to 14 mT EMF (5–100 Hz), no changes were observed [14]. Therefore, these authors conclude that there is no evidence that magnetic field could act as a stress factor altering basic physiological mechanisms.

Nowadays, the number of papers published concerning the bioeffects of EMF has been increasing with conflicting results obtained in different laboratories, probably because of the use of different cell lines and exposure protocols. This situation leads us not to be able to formulate hypotheses that explain the effects observed in some laboratories.

The discordant results published from different laboratories demonstrate that the problem is still unresolved. This is the base to establish exposure conditions that let us determine the physical and biological parameters influencing the EMF bioeffects. In this way, the aim of the present study was to investigate whether long-term and continuous exposures to static or 50 Hz sinusoidal MF produce alterations in the growth of *S. cerevisiae*.

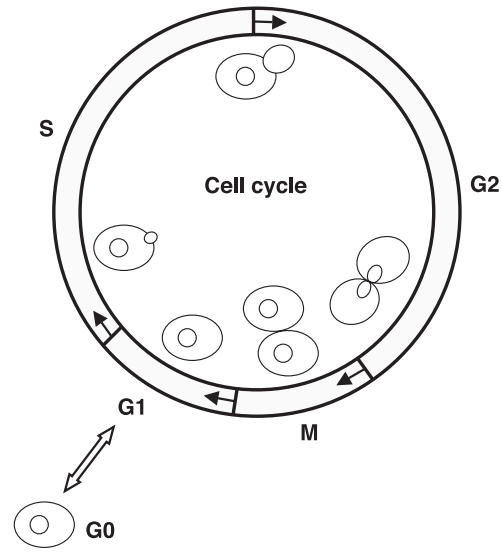

Fig. 1. Cell cycle of *S. cerevisiae*. Exponentially growing cultures contain cells in all stages of the cell division cycle (G1, S, G2, M). This yeast divides by budding, being the emergence of the bud indicative of the end of G1 and the beginning of the S phase. Both G2 and M phases include cells with large buds and G0 cells are indicative of resting cells not actively cycling.

2. Materials and methods

2.1. Yeast strain and culture medium

The experiments were carried out with the haploid yeast strain *S. cerevisiae* WS8105-1C (genotype: *MATalpha, ade2, arg4–17, trp1–289, ura3–52*) [15,16]. It was supplied by Dr. Anna A. Friedl (Radiobiological Institute, Ludwig-Maximilians-Universität, Munich, Germany). Yeast cells were grown in a medium of YPD broth (1% Bacto-yeast extract, 2% Bacto-peptone, 2% dextrose) with or without 2% Bacto-agar.

2.2. Experimental protocol

For the purpose of this work, we decided to use undistributed cells growing exponentially while exposed to MF. Such a population contains cells in all stages of the mitotic cell cycle (G1, S, G2, and M; Fig. 1).

Prior to exposures, yeast cells were cultured for 3 days on YPD-agar plates at 30 °C and then suspended in YPD broth at a titer of $1.13–2.24 \times 10^7$ cells/ml, depending on the incubation loop transferred into the medium. An equal number of cells from the same culture was used simultaneously in each experiment for each MF-exposed and -unexposed control sample. Cell suspensions in ependorf tubes were continuously incubated at control location and in the exposure system, at room temperature (23 °C). All incubations and exposures were done in the dark. Exposed cells were treated with static and 50 Hz sinusoidal MF, at 0.35 and 2.45 mT. The background magnetic field in the room at control location was 0.035 mT. At various times after MF exposure (24 and 72 h), cells were collected for growth measurements.

2.3. Exposure system

Magnetic fields are generated by a pair of Helmholtz coils (40 cm in diameter) with 154 turns of copper wire in each, mounted on a wooden frame. The coils were separated 20 cm and produce a homogeneous field in the vertical direction in the central area near the axis of the coils (Fig. 2). Yeasts were located in the region within the coils where fields are homogeneous to better than ± 5%. The induced mean MF in the centre of the coils could range from 0.1 to 3.5 mT. Magnetic field excitation waveforms and amplitudes were monitored by an oscilloscope (Hameg HM 204), and field intensity was measured by a Digital Teslameter (Phywe España, Madrid, Spain) by means of an axial probe (Hall effect). The Teslameter accuracy is ± 2% for DC and ± 3% for AC. Both samples and controls experienced the same sequence of manipulation, temporal coincidence of treatment, and equal ambient conditions (23 °C). The control cultures were located at a 5-m distance from the coils. The experiments were performed at 0.35 and 2.45 mT, DC, and 50 Hz sinusoidal.

2.4. Growth measurements

The growth of the MF-exposed and control samples were monitored by measuring the optical density at 600 nm.

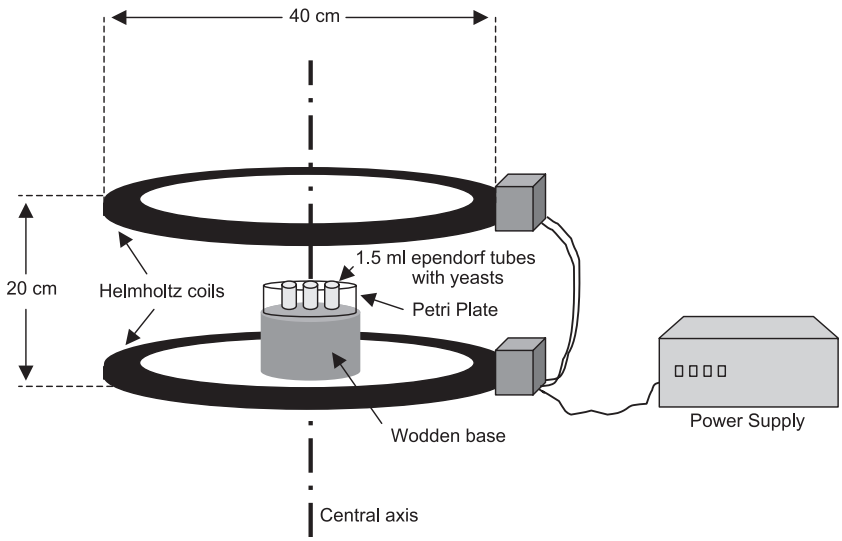

Fig. 2. Magnetic field exposure system. The magnetic fields were generated by a pair of Helmholtz coils that produce a vertical homogeneous magnetic field in the central area near the axis of the system where the yeasts were located.

2.5. Statistical analyses

The Wilk–Shapiro rankit plot test was used to assess the normal distribution of the data. Additional statistical analyses were made with the Student's *t*-test. Differences were considered significant when $p < 0.05$.

3. Results and discussion

As shown in Fig. 3, the static or 50 Hz sinusoidal MF exposures (0.35 or 2.45 mT) during 24 and 72 h did not induce alterations on the cellular growth of exponentially growing yeasts in relation to unexposed control cells, as

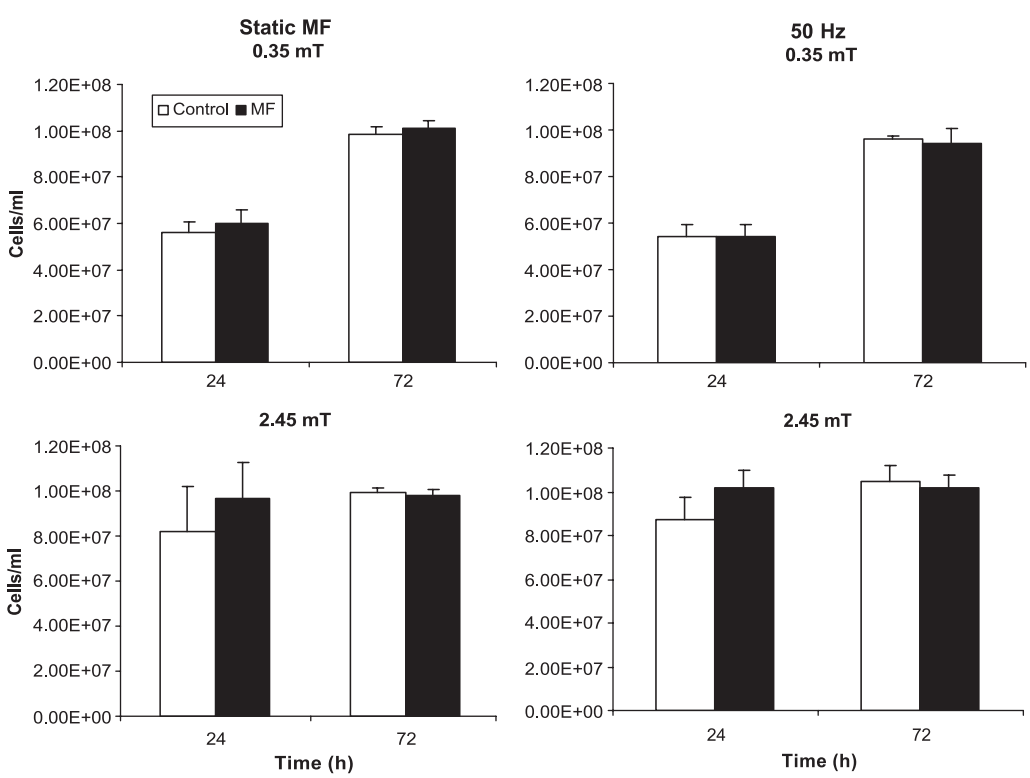

Fig. 3. Effect of static and 50 Hz magnetic fields (MF) on the growth of *S. cerevisiae*. Exponentially growing cells were exposed to magnetic fields during 24 and 72 h. Mean ± S.D. from two to six independent experiments. $p > 0.05$ Student's *t*-test.

measured via the optical density of the samples at 600 nm; $p>0.05$ Student's *t*-test.

These results are in disagreement with those published previously by Mehedintu and Berg [11], and they do not follow the line of results obtained by Motta et al. [12] for the lower field used in this paper.

It is generally shown in the papers published during the last decade that there is difficulty in replication experiments related to MF effects. This situation could lead us to think that not all the influencing parameters (physical and biological) are being taken into account in experimental studies.

Laboratory studies have the advantage of the facility to control the parameters that could influence the experiment, but when we do not know what the total number of influencing parameters is, it is easy not to make an exhaustive control of all of them. This circumstance could be the reason of the difficulty in replicating the experimental studies in other laboratories. The physical parameters of the MF (intensity, frequency, time of exposure, waveform, etc.), the cell type, the temperature of exposition, and the biological state of the cells could influence the possible effects of MF on biological systems. Under laboratory conditions, these parameters can be easily controlled, which represent an important advantage in relation to epidemiological studies; therefore, it would seem easy to replicate the experiments performed in other laboratories.

Therefore, it is very important to perform a complete control of the parameters that could influence, making an exhaustive characterization of the MF, exposure protocol, manipulations of samples, temperature of exposition, time intervals before and after exposures, and what could be one of the most important parameters, the biological state of the cells, without forgetting the MF waveform.

When a yeast strain is exposed to a physical agent, it is expected, in general terms, that the agent acts as a stress or cytotoxic factor inducing cell damage or in contrast resulting in no effect to the cell. In this way, it is well known and documented that wild type yeasts have very efficient repair mechanisms to avoid the damage induced by physical and chemical agents even at high doses [16], which are not according to the findings described previously by Mehedintu and Berg [11]. In addition, it is expected that *S. cerevisiae* wild type strains respond, in different laboratories, in the same way to physical and chemical agents. The repair processes in yeast could be a problem when we want to study the possible bioeffects of EMF. Nevertheless, the facility to obtain specific yeast mutants in specific genes which could not repair the induced damage could be an important tool in the study of the possible interaction or damage of EMF with different basic physiological processes, which otherwise in wild type cells would be hidden by reparation. There have been published very few articles concerning the use of yeast strains. Thus, it is expected that the use of well-characterized mutant strains and the performance of experiments that control all the physical and biological influencing parameters will be increasing in further studies.

In conclusion, the data presented in the current report indicate that static and 50 Hz sinusoidal MF (0.35 and 2.45 mT) do not induce alterations in the growth of *S. cerevisiae*.

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