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## Effect of magnetic fields on the growth of bacterium *Staphylococcus aureus*

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### Abstract

The effects of weak static and low-frequency magnetic fields on the growth rate of *S. aureus* were investigated. The measurements were performed with static magnetic fields with magnitude in range  $0.0 \text{ G} \leq H_0 \leq 14.0 \text{ G}$  and low-frequency magnetic fields at fixed amplitude  $H_0 = 4.5 \text{ G}$ , and frequency range  $0.0 \leq f \leq 1.0 \text{ kHz}$ . The growth of these bacteria is negatively affected by increasing the intensity of the static magnetic field. When exposed to an oscillating field, a positive effect was observed on the rate of growth of the colonies with respect to the field frequency. In both, static or ac magnetic field, the growth curves follow an exponential law, typical of asynchronous cell divisions.

**Keywords:** Asynchronous growth; exponential growth; magnetic field effect on microorganisms; *S. aureus*.

## Efecto de campos magnéticos en el crecimiento de la bacteria *Staphylococcus aureus*

### Resumen

Los efectos de campos magnéticos estáticos y de baja frecuencia sobre la tasa de crecimiento de *S. aureus* fueron investigados. Las medidas fueron realizadas con campos magnéticos estáticos con magnitud en el rango  $0.0 \text{ G} \leq H_0 \leq 14.0 \text{ G}$ , y bajas frecuencias en el rango de  $0.0 \leq f \leq 1.0 \text{ kHz}$ , con amplitud de  $H_0 = 4.5 \text{ G}$ . La tasa de crecimiento de esta bacteria disminuye al incrementar la intensidad del campo estático. Sin embargo, en presencia de un campo oscilante de baja frecuencia, se observa un efecto positivo en la tasa de crecimiento respecto de la frecuencia del campo. En ambos campos, estático y variable, las curvas de crecimiento satisfacen una ley exponencial, típica de una división celular asíncrona.

**Palabras clave:** Crecimiento asíncrono; crecimiento exponencial; efecto de campos magnéticos en microorganismos; *S. aureus*.

## Introduction

Nowadays the exposure of living tissue to various types of electric and magnetic fields is a commonly encountered event: extremely low frequency from power lines, high frequency electromagnetic fields (EMF) from cellular phones, and computers. Since this is a task of medical and technological importance, a number of attempts have been given to clarify the effects of electric and magnetic fields on biological cells<sup>1</sup>.

*S. aureus* is a human pathogen responsible for a variety of community-acquired diseases, belonging to the class of gram-positive bacteria <sup>2</sup>. With the spread of this bacterium, the number of antibiotic agents has increased and along with these, stronger antibiotic resistance profiles have been observed <sup>3, 4</sup>. This requires new and more efficient methods for treating infections. One of the techniques used for medical purposes is the magnetic field therapy or magnetotherapy <sup>5</sup>, which is often applied in the treatment of many diseases such as bone fractures <sup>6</sup>, pain syndromes <sup>7</sup>, and cancer <sup>8</sup>. Also, pulsed magnetic field-based methods are also employed as non-thermal preservation techniques to minimize the risk of microorganism contamination <sup>9, 10</sup>. This is because of the proven ability of oscillatory fields to cause damage in living cells. However, the effects are not fully understood, since some of the results have been inconsistent <sup>11</sup>. In other cases the results often contradict each other, which include an increase or decrease in the rate of cell division in *E. coli* and *S. aureus*, when these strains are in presence of a magnetic field <sup>12-14</sup>. Some other studies found that magnetic fields could be a general stress factor in bacteria <sup>15</sup>. The general stress response to a magnetic field is found in all bacteria, and living cells and is remarkably conserved across specie. In a study on the mutagenicity of magnetic fields exposure, Ikehata <sup>16</sup> also reported that strong static magnetic fields can cause mutations in *S. typhimurium* and *E. coli*.

In this work we study the effects of weak static and low-frequency magnetic fields on the growth of bacterium *Staphylococcus aureus*. From an analysis of the growth curves, we have found that the main effect of the magnetic field on the growth dynamics of *S. aureus* is to affect the time required for the cell divisions.

## Experimental

Fresh *S. aureus* strains were used throughout the experiments. Nutritive Broth (Merck, Darmstadt) and Plate Count Agar (Difco, Detroit) were used for cultivation of the bacteria. Salt solution 0.75% was used to make serial dilutions until  $10^{-5}$  ml. The control cultures were kept in the same conditions as the exposed ones except the sole exposition to the magnetic fields. The number of colonies forming units (CFU) of the bacterial cultures was measured independently as a function of the magnetic field intensity ( $H_0$ ), and frequency ( $f$ ).

The magnetic fields were generated by a homemade 600 turned cylindrical coil (12 cm radius and 30 cm length), and were measured by a Hall effect probe Gaussmeter. Two different experiments were performed: (a) the cells were exposed to static magnetic fields with amplitude varying from 0.0 to 14.0 G and (b) with the cells exposed to oscillating magnetic fields with frequencies ranging from 0.0 Hz 1.0 KHz and fixed intensity of the order of 4.5 G. The magnetic fields inside the solenoid were approximately homogenous in a region  $\pm 3$  cm off the center of the coil. The device was kept at 37°C in an incubator cabinet and it was measured by a thermometer.

The samples were placed first into glass tubes on a nonconductive stand (homemade) along the axis of the coil, and then introduced inside the solenoid during exposure times from 0 h to 6 h. In order to reduce the uncertainty in our measurements and to obtain reliable results, each test was performed independently up to 4 times keeping the same experimental conditions.

## Results and discussion

### 1. Effect of static magnetic fields

The main effect of the static magnetic field on the growth dynamics of the bacterium *S. aureus* is shown in Fig. 1. Each symbol is an average from 4 independent measurements performed previously. We found that the number of CFU increases with the time of exposure and decreases with the magnitude of the applied field.

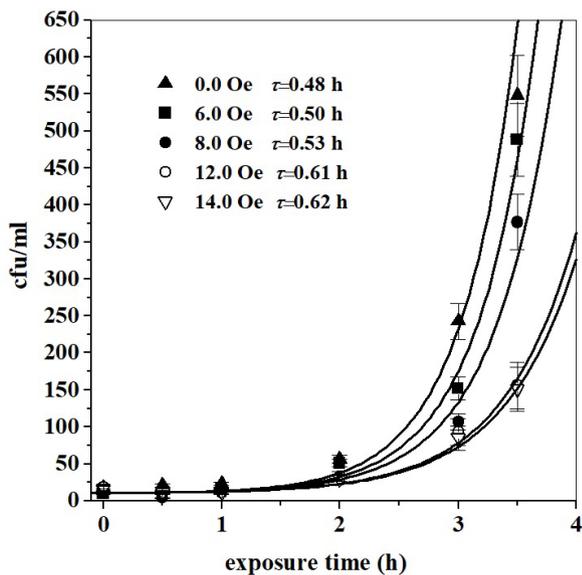


Fig. 1. Dependence of the colony forming units (CFU) on the time of exposure for several magnetic field intensities. The solid curves are fits to the exponential function  $e^{t/\tau}$ , as described in the text. The curve for  $H_0=0.0$  G (▲) correspond to the control culture.

These results are in agreement with previous studies which also found the magnetic field intensity negatively affects the growth of *S. aureus*<sup>12</sup>. It is also observed that the cells remain in lag state up to certain time of exposure, after which, cell division is activated growing in an exponential trend. To estimate the quantity  $\tau$ , we assume that the growth dynamics is governed by some mechanism following the exponential law  $et/\tau$ , where  $t$  is the exposure time and  $\tau$  is related to the time required for a cell division, and is an intrinsic parameter to the species in its environment. These functions are commonly used to describe asynchronous growth in population dynamics<sup>17</sup>. In an asynchronous process, the division might occur at different times in each cell. The solid curves in Fig. 1 represent exponential functions with  $\tau = 0.48, 0.50, 0.53, 0.61,$  and  $0.62$  hours, for  $H_0 = 0.0, 6.0, 8.0, 12.0$  and  $14.0$  G, respectively. These results are plotted in Fig. 2, and show that as the magnetic field intensity is increased the growth process is decelerated, but not stopped. The dashed line is a guide to the eyes.

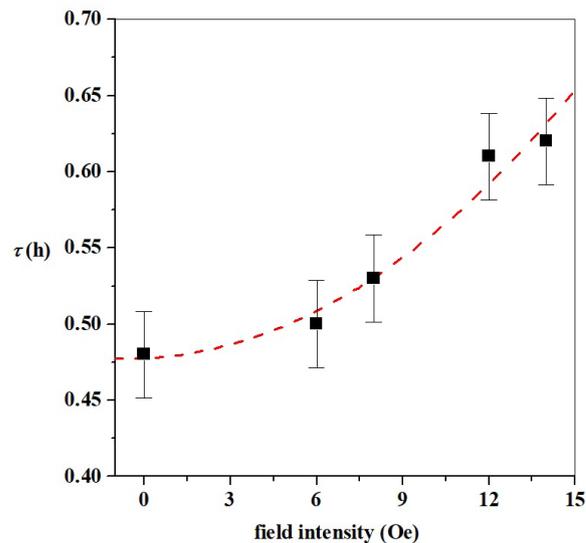


Fig. 2. Activation time,  $\tau$ , as a function of the magnetic field strength. The dashed line is to guide to eyes.

Although the dc magnetic field decreases the number of colony forming units, is it not obvious that the cells loose their ability to divide. This means that the inhibitive effect of the static field is not fully bacteriostatic in this range of magnetic fields. Since the growth curve of the control culture ( $H_0 = 0.0$  G) is also exponential, we conclude that the asynchrony in the cell division is inherent of the preparation conditions, and independent of external agents such as a dc magnetic field.

## 2. Effect of low frequency magnetic fields

When exposed to an oscillatory magnetic field, bacteria can behave unexpectedly. To study this behavior, trends of *S. aureus* were grown *in-situ* in presence of a magnetic field with frequency ranging from 0.0 Hz to 1.0 kHz. After an exposure time of about 6.0 h, we counted the number of CFU of the exposed cultures and compared with the control ( $f=0.0$  Hz). The resulting growth curves of *S. aureus* are shown in Fig. 3 for several frequencies. As in the case of the dc magnetic field the CFU increase exponentially with exposure time, but on the contrary, it increases rapidly with increasing frequency. These curves are adjusted to exponential functions of the type  $e^{t/\tau}$  (solid lines), with  $\tau$  values depending monotonically on the frequency of the magnetic field, as shown in Fig. 4. The dotted line is a guide for the eye. This fact can be used to understand the dynamics of the bacterial growth.

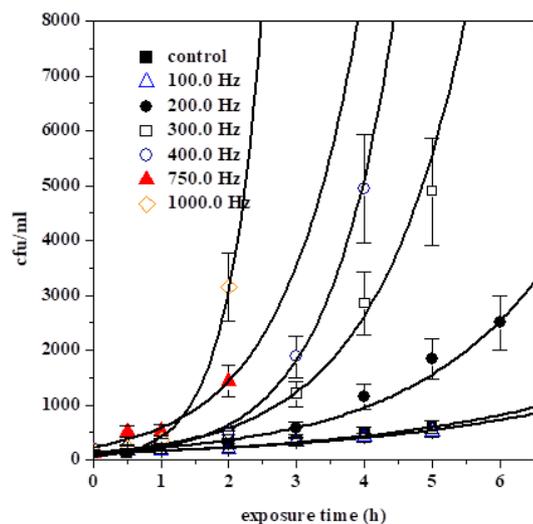


Fig. 3. Dependence of the colony forming units (CFU) on the time of exposure for several frequencies. Solid lines are fits to the exponential function  $e^{t/\tau}$ , as described in the text. The curve for  $f = 0.0$  Hz (■), correspond to the control culture.

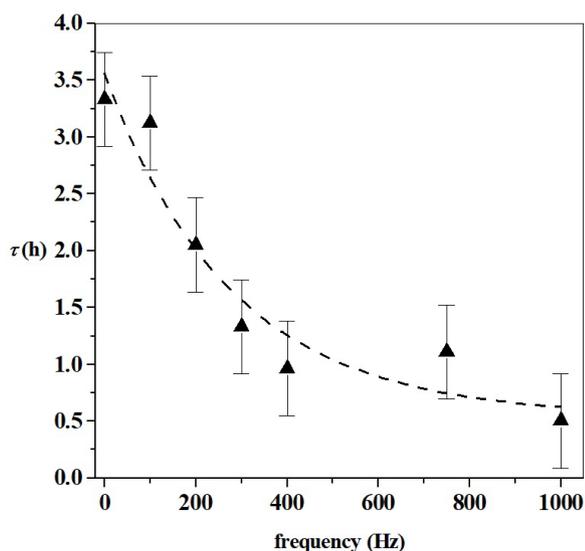


Fig. 4. Activation time,  $\tau$ , as function of the frequency of the ac magnetic field. The dotted line is a guide for the eye.

According to these results, the growth dynamics in *S. aureus* cultures is governed by a synchronous frequency-independent mechanism, and the effect of frequency is to accelerate the cell division process. Our results indicate that the weak static field of a few G after a few hours give a measurable change in the growth rates of all of the bacterial species and are in agreements with previous works<sup>18,19</sup>.

## Conclusions

We have presented and discuss and experimental investigation on the effect of dc and ac magnetic fields on bacterium *S. aureus*. It was found that as prepared, the growth dynamics is governed by a synchronous mechanism. Although this mechanism is qualitatively unaffected by the magnetic field, the rate of growth decreases for increasing the field intensity, and increases for increasing the field frequency. We have determined that the activation time for the cell division depends on both magnitude and frequency of the applied field.

The question of how the growth dynamics of bacteria is affect by a magnetic field is not completely answered, and still an open issue. The fact that the activation time for a cell division can be expressed as a function of the magnitude and frequency of the magnetic field, can be important to understand metabolic changes due to ion transport across the cell membrane.

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