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Low-Level, Magnetic-Field-Induced Growth Modification of *Bacillus subtilis*

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Experimental studies showed an increase in the growth of *Bacillus subtilis* mutant strain FJ7 above controls by exposing the bacterial culture to 800-Hz or 1-KHz magnetic fields with a 2-s-on /2-s-off period. The magnetic field strength was between 0.8 and 2. 5 mT. Light microscopy and scanning electron microscopy demonstrated the morphology of controls to grow in a macrofiber of right-handed helix formation. In contrast, the field-exposed group showed little to no cohesion; the cells appeared to be homogeneously distributed throughout the sample. These results suggest that growth patterns of *Bacillus subtilis* can be altered as a result of magnetic-field-induced effects.

Key words: ELF, field effects, bacteria, growth enhancement, pulsed magnetic fields

INTRODUCTION

In this communication we report the frequency- and field-strength-dependent behavior of the growth and morphology of *Bacillus subtilis* when exposed to low-intensity sinusoidal magnetic fields. We used a strain of *Bacillus subtilis*, FJ7, C-type mutant, which is characterized by a tendency to form macrofibers in a right-handed helix [Mendelson, 1976]; thus, some changes in growth could be detected as changes in the helical morphology. Several frequencies in the range of 60 Hz to 1 kHz were tested, and it was found that growth of *Bacillus subtilis* could be enhanced significantly above controls when exposed to sinusoidal fields at 800 Hz and 1 kHz. Fifteen to 76% enhancement in the growth of exposed samples relative to control samples was observed by increasing the field strength from 0.8 mT (8 G) to 2.5 mT (25 G) at frequencies of 800 Hz and 1 kHz. We also tested several wave shapes and on-off periods and found that growth of exposed samples above the controls was maximum with a 1-kHz sinusoidal signal with the field switched on and off every two seconds; this corresponds to a 1 kHz signal modualted by a 0.25-Hz square wave. Due to the nonlinear distortion of signal in the magnetic coils, we did not collect data above 1 kHz; therefore, the dependence of the growth curve at higher frequencies cannot be determined from this study. We also found a greater enhancement in the growth with a periodic on-off field than when a continuous sinusoidal field was applied.

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Light microscopy of the exposed and control samples were also performed which showed control bacteria to grow in a macrofiber with a right-handed helix, while exposed bacteria showed very little adhesion of adjacent cells, producing an even distribution of the bacterial cells. Both samples, control and exposed, were grown without shaking for microscopy examination. Details of scanning electron microscopy confirmed light microscopy results.

A literature search does not show any previous work done on the effect of low-level magnetic fields on the growth of *Bacillus subtilis* except one study by Moore [1979], which employed a higher-strength, 0.3-Hz pulsed magnetic field of 15 mT to 60 mT. After exposure to the pulsed magnetic field for 4 h, it was found that there was slight decrease in the cell count as compared to the controls at 30-mT and 60-mT magnetic fields and no observable effects at a 15-mT magnetic field. No attempt were made to examine the exponential growth phase of *Bacillus subtilis* under the influence of the magnetic fields. Results reported here will be difficult to compare with Moore's [1979] data because of differences in the field strength and, in particular, frequency of the applied magnetic field; 0.3 Hz used by Moore [1979] as compared to 800 Hz and 1 kHz used here.

MATERIALS AND METHODS

The experimental arrangement consisted of paired resonant Helmholtz coils capable of producing a uniform field in a volume sufficient to accommodate bacterial cultures in test tubes. Each coil was 16 cm in diameter, 1.6 cm in width and was made of 250 turns of 18-gauge insulated copper wire. Both coils were kept parallel and vertical on the opposite sides of a rectangular water bath. Thus, the field lines were horizontal. Axial separation between the coils was 7.5 cm and coils were not in physical contact with the water bath. The water was circulated and kept at a constant temperature of 32.8°C $\pm$ 0.1 °C by use of a heater/water pump (Polyscience Corporation). The water temperature was monitored near the exposed and control samples. A schematic arrangement of the setup is shown in Figure 1. The field strength as measured by a gaussmeter (F.W. Bell model 610) was found to be uniform within $\pm$ 0.1 mT variation within the working volume of 2.5 cm $\times$ 2.5 cm $\times$ 5 cm. The field coils were powered by a function generator (Exact model 517) and a power amplifier (Crown model 7520). A variable capacitor was connected in parallel with the field coils for maximizing the current flow in the capacitor-inductor loop; the measured value of the inductance of the coils was 15 mH. The field coils produced a very low intensity acoustic tone when 800 Hz or 1 KHz signal was applied. The tone was due to the mutual attraction of the current carrying wire in the field coil and was reduced by tightly winding the coil. The intensity of the acoustic tone was measured by a sound level meter (Quest Electronics, model 155) and was 41, 46, and 51 db when the magnetic field produced by Helmholtz coil was 0.8, 1.6, and 2.5 mT, respectively. This is a low-intensity acoustic tone generated by the coils, but, in order to isolate the effect of magnetic field on the growth, the control samples were also exposed to the same strength acoustic tone but shielded from the magnetic field. Magnetic coils were connected in paralles and powered by one channel of the power amplifier. The other channel of the power amplifier was utilized to power the water-immersible miniature speaker to produce the acoustic tone for the control samples. Power level in both channels could be independently varied.

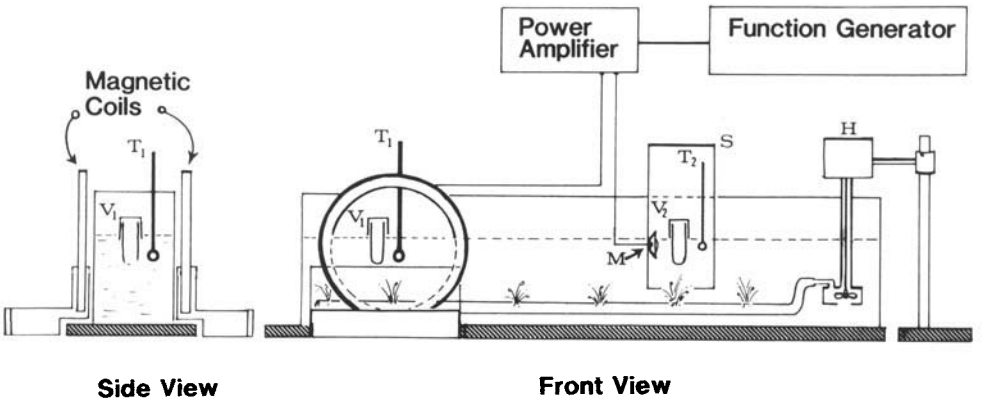

Fig. 1. Schematic diagram of experimental setup. Drawings not to scale. Front view showing details of exposure system, mu-metal shielded area for control samples, and water circulator and heater. Side view showing details of the mounting arrangements for magnetic field coils. H is heater/water pump; M is miniature water immersible speaker; S is mu-metal magnetic shield; T_1 and T_2 are thermometers; V_1 and V_2 are bacterial samples in vials. A group of five vials were used for exposure to magnetic fields and five vials were used as control. For simplicity only one vial at the location of exposure and control positions is shown.

The noise spectrum of the circulating water in the bath was also measured by use of sound level meter described above a real-time spectrum analyzer (Unigan, model 4512) utilizing fast Fourier transforms. Most of the noise was confined to frequency range of 10–250 Hz. Discrete noise measurements at 31.5, 63, 125, and 250 Hz were 42, 51, 62, and 57 db, respectively. Noise level above 250 Hz gradually tapered off, falling below 30 db at frequencies above 500 Hz.

Aliquots of 10 ml of trypticase soy broth, pH 7.2, were placed in glass vials and inoculated with a 20-h-old bacterial culture grown separately in trypticase soy broth. Ten inoculated samples were divided into two groups of five samples each. One group was placed between the coils for exposure to the magnetic field. The control group was placed 35 cm from the coils inside a mu-metal cylinder to further shield them from the magnetic field. The control group was exposed only to an acoustical tone identical in intensity and waveshape with that produced by the field coils. Both control and exposed samples were placed simultaneously in the same water bath at the start of the experiment and also grew simultaneously in the same water bath during the course of each experiment. The magnetic field was measured inside the shield for DC to 1-kHz frequency by use of a digital magnetometer (Model DM 2220 of Schnostedt Instrument Company), and was less than 0.001 mT, which is significantly less than the field strength of 0.8–2.5 mT used for the magnetic-field exposed group.

The bacterial cell count was measured by using a Klettmeter which functions by monitoring the intensity of transmitted light through the bacterial sample in a test tube. Each vial of the cell culture was vigorously shaken for 1 min, and then the cell growth was measured. This was done to reduce error due to the formation of macrofibers in the control sample and disaggregated bacteria in the field-exposed samples. The *Bacillus subtilis* FJ7, mutant tends to form helical macrofibers only when left without shaking during the growth cycle. Vigorous shaking every few hours for Klettmeter reading, as done here, considerably reduces the tendency to form macrofibers. In light microscopy examination, it was found that macrofiber structure

was not present in exposed or control samples which were vigorously shaken. A red transmission filter centered at 650 nm was used in the Klettmeter. The mean value and standard deviation of transmittance was calculated for each group containing five samples each. The difference between control and exposed samples was considered statistically significant at $P < .05$. The Student's t-test was used for all statistical analysis.

Several pilot experiments were performed before the actual data collection in order to monitor the temperature inside the glass vials as compared to temperature of circulating water, to examine the effect of mechanical vibrations of the magnetic coils, and to assess the effect of the acoustical noise at 800 Hz and 1 kHz on the bacterial growth. The temperatures of the bacterial sample inside the glass vials and the circulating water in the vicinity of the glass vials were monitored by use of thermometers. It was found that the temperature inside the glass vials stabilizes to the outside circulating water temperature with variation of $\pm$ 0.1°C within 0.5 h. after initially placing the vials in the water bath. Temperatures of the control and exposed samples inside the glass vials were also monitored when the magnetic field was on and were found to be 32.8 °C+ $\pm$ 0.1 °C, which is the same as the temperature of the circulating water in the bath. Since the magnetic field was not heating the bacterial samples, the temperature of the circulating water was monitored in later experiments in the vicinity of the exposed and control samples by use of thermometers as shown in Figure 1. Growth of *Bacillus subtilis* was monitored in two separate experiments, one in which the magnetic field coils were rigidly attached to outside walls of the water bath and the other experiment in which the coils were not in physical contact with the water bath. No significant difference ($P > 0.05$) in the growth curves was found, which demonstrated that the low-strength magnetic field used in experiments here does not produce large enough mechanical vibrations in the magnetic coils to influence growth rate. Nevertheless, the data reported here was taken when the magnetic coils were not in contact with the water bath in order to definitely isolate the effects of the magnetic field on the growth of *Bacillus subtilis*.

Pilot experiments were also performed to separate the effects of the acoustic tone and the magnetic field plus acoustic tone on the growth of bacteria. It was done by simultaneously taking three sets of data on the growth of bacterial samples. One set of bacterial samples were exposed to the magnetic field and accompanying acoustic tone, a second set were exposed to the acoustic tone only, and a third set were shielded from magnetic field and the acoustic tone by use of a styrofoam lined mu-metal cylinder. Residual noise inside the shield was below 15 db at 1 kHz frequency. Each set consisted of five vials of bacterial sample, a total of 15 samples. All samples were kept in a water bath at 32.8 °C $\pm$ 0.1 °C. For a 0.8-mT magnetic field at 1 kHz with 2-s-on/2-s-off period, the growth was higher for samples exposed to the magnetic field as compared to the totally shielded samples, and the growth was decreased for samples exposed to the acoustic tone only as compared to the toally shielded controls. This experiment was repeated and identical results were found. In the middle portion of the growth cycle, after 13 h of exposure, the growth enhancement of samples exposed to the magnetic field plus acoustic tone was 23% compared to totally shielded control samples, while reduction in the growth of the samples exposed to the acoustic tone only was 10% compared to the totally shielded control samples.

Based on these pilot experiments where opposing effects of magnetic field and acoustic tone on the growth of *Bacillus subtilis* were found, it was decided, as

described earlier, to expose the control samples to the same intensity acoustic tone as generated by magnetic coils by use of a miniature water immersible speaker.

Gram staining was performed for comparison with the original culture. Wet mounts of the bacterial culture (postfixed) were prepared for light microscopy examination after 20 h of field exposure without shaking. The magnetic field strength was 0.8 mT, 1 kHz with a 2-s-on/2-s-off period. Control cultures shielded from magnetic field were also grown for 20 h without shaking. For scanning electron microscopy work, the exposed and control cultures, 20 h after inoculation, were centrifuged for 20 min at 1,000 rpm and resuspended in veronal acetate buffer with a 1% osmium tetroxide fixative. Cells were washed twice and then vacuum fixed on a nucleopore filter. A Japan Electron Optical Laboratory, model JSM-35U, scanning electron microscope was used to obtain photomicrographs.

RESULTS

A typical growth curve is shown in Figure 2 at a field strength of 0.8 mT at 1 kHz frequency with a 2-s-on/2-s-off period. The curves are labeled and refer to bacterial cultures exposed to the magnetic field plus acoustic tone, and the control group shielded from the magnetic field but exposed to acoustic tone. More experiments with similar protocols were performed to measure the growth curves for field strengths of 0.8, 1.6, and 2.5 mT 800 Hz and 1 kHz sinusoidal signal. All of these growth curves which are not shown here were similar in shape to that shown in Figure 2.

It is apparent from the growth curves in Figure 2 that the magnetic field enhances growth of exposed samples compared to the controls. From Figure 2 one notices that not only is the growth of exposed samples higher than controls at mid log phase but so is the final stationary concentration after 25 h of exposure to the magnetic

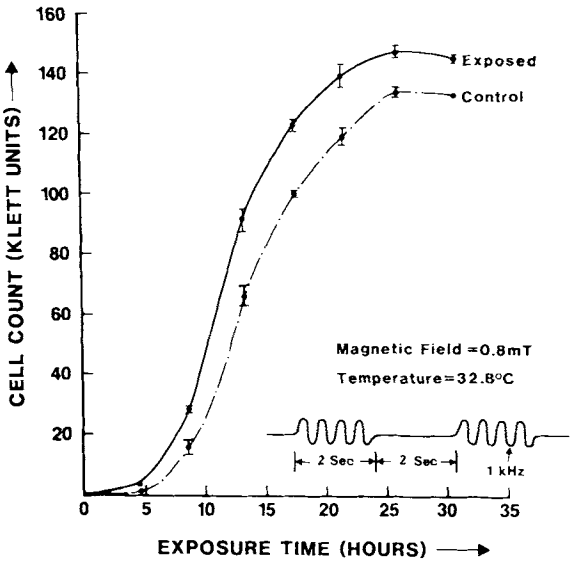

Fig. 2. Growth curves of *Bacillus subtilis* exposed to 0.8-mT magnetic field plus 41 db acoustic tone at 1-kHz sinusoidal signal with 2-s-on/2-s-off period. Control cultures were shielded from the magnetic field but exposed to the same intensity acoustic tone. Growth curves are based on mean and standard deviations of five samples at each data point.

field. Also, note the shorter lag phase of the exposed samples compared to controls. Similar growth behavior was observed in all of the experiments at different frequencies or at different field strengths.

To compare several experiments, we measured the magnetic-field-induced growth enhancement at the midpoint of the growth cycle, ie, approximately 13–14 h postinoculation. Growth enhancement is defined as follows:

$$\% \text{ Growth enhancement} = \frac{\text{transmittance exposed} - \text{transmittance control}}{\text{transmittance control}} \times 100 \quad (1)$$

Growth enhancement was 38% ± 3% (SEM) for n = 5 (Fig. 2). Growth enhancement in the middle portion of the growth cycle, as defined above, was calculated for each experiment and tabulated in Table 1.

Table 1 lists the growth enhancement of exposed samples above controls at the midpoint of the log phase cycle for field strengths of 0.8, 1.6 and 2.5 mT at two sinusoidal frequencies, 800 Hz and 1 kHz. There are three entries in the table, 800-Hz continuous, 800-Hz on-and-off and, 1-kHz on-and-off. Growth enhancement is highest with a 1-kHz on-and-off signal. The table also shows that the 800-Hz signal gives better growth enhancement when switched on and off than with the continuous 800-Hz signal.

Figure 3A, B shows samples by Namarski interference comparing the control and field-exposed group after 20 h of growth without shaking the vials. Figure 3C,D shows scanning electron micrographs of the control and field-exposed groups. The magnetic field strength was 0.8 mT at 1 kHz with a 2-s-on/2-s-off period. Light and scanning electron microscopy pictures in Figure 3 show a definite change in the growth patterns. There is a growth of field-exposed bacteria with little or no aggregation of adjacent bacteria. The characteristic macrofiber formation was missing in the field-exposed group, as contrasted with the control samples, which showed considerable fiber formation.

DISCUSSION

Growth curves in Figure 2 and microscopy pictures in Figure 3 clearly demonstrate that low-frequency weak magnetic field can both enhance the growth of *Bacillus*

TABLE 1. Percentage Growth Enhancement of *Bacillus subtilis* Above Control Samples at the Midpoint of the Logarithmic Phase of the Growth Cycle at Different Field Strengths of 800-Hz and 1-kHz Sinusoidal Field Signals*

	% Growth enhancement above control		
Field strength (mT)	800-Hz continuous (n=5)	800-Hz pulsed (n=5)	1-kHz pulsed (n=5)
0.8	15 ± 2	33 ± 3	38 ± 3
1.6	20 ± 3	44 ± 4	50 ± 4
2.5	22 ± 2	54 ± 3	76 ± 5

*See text for the definition of the growth enhancement. Standard error of mean is based on five samples. Control and exposed samples were statistically different ($P < .05$) as determined from Student's t-test. Pulsed signals were with 2-s-on/2-s-off duty cycle.

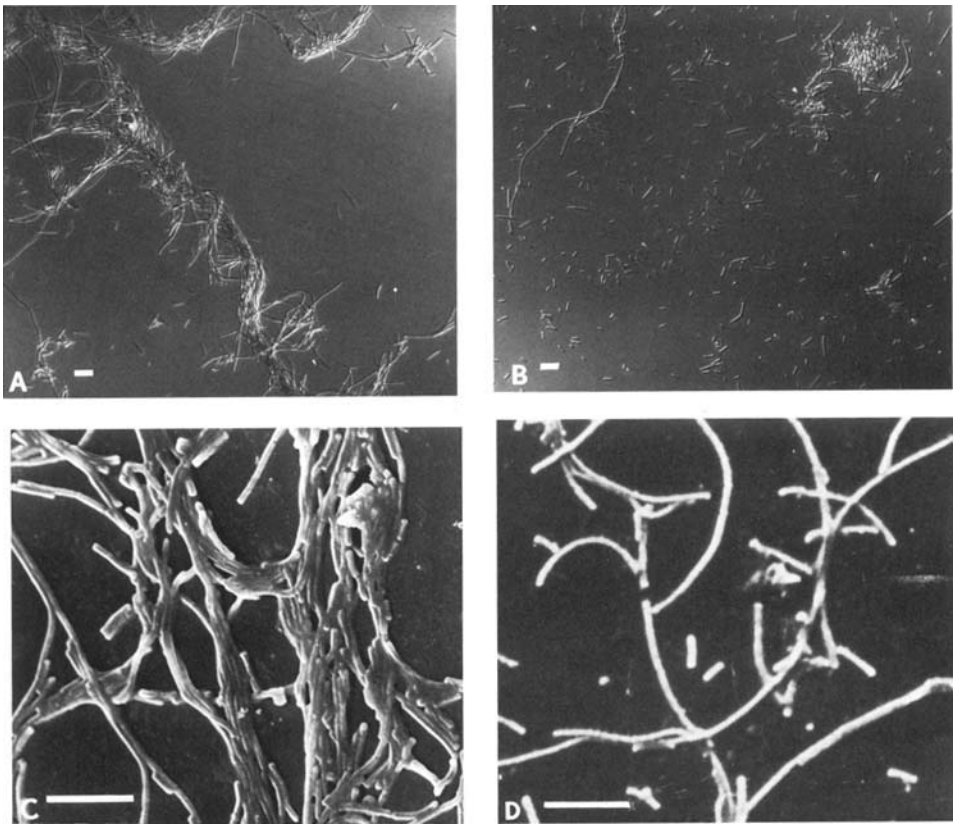

Fig. 3. Photomicrographs by Namarski interference of *Bacillus subtilis* FJ7 mutant. Original magnification, ×400. **A**: Control. **B**: Postexposure to magnetic field for 20 h. The magnetic field strength was 0.8 mT with a 2-s-on/2-s-off period. Scanning electron micrograph of same bacteria. ×2,000. **C**: Control. **D**: Field-exposed. Note the macrofiber right-handed helix formation in the control and the absence of it in the field-exposed samples. Calibration line = 10 μm.

subtilis above controls as well as destroy the morphological macrofiber helix formation. Dose response based on the strength of the magnetic field, frequency, and on/off period of the field is also observed and the frequency dependent effects can be partially explained by use of recent theorectical studies of Drago et al [1984]. They have described a model of a cellular structure applicable to the effects of electromagnetic fields. Their computed results show that various cellular parameters, eg, ionic charge densities on cell membrane, current density within the cell, and voltage across the cell membrane are frequency dependent quantities which can be optimized by selecting proper frequencies of applied electromagnetic fields. In particular, 0.25 Hz falls within this range of frequencies where ion-density variations, passive ion flux, and absorbed ion densities are maximized. We postulate that this could be the reason for greater enhancement in the growth rate with a periodic on-off field than with a continuous sinusoidal field. We found in trial experiments that acoustic noise suppresses growth while magnetic field enhances growth of bacteria. The noise spectrum of the circulating water is confined to the frequency range of 10–250 Hz with maximum acoustic noise of 62 dB at 125 Hz. This could be the main reason that we

did not observe any significant growth enhancement of *Bacillus subtilis* owing to the magnetic fields at lower frequencies.

It has been found that there is a calcium ion effux under the influence of modulated low-frequency electromagnetic fields [Bawin et al, 1975]. Calcium ions play a large role in cell membrane transport and regulatory function [De Vrij et al, 1985], and the binding of calcium ions to proteins in the phospholipid bilayer could modify these properties. A change of calcium binding would cause a modification of adsorbed ion concentration which may be the cause of less aggregation of adjacent cells as observed in the field-exposed samples.

In conclusion, this study demonstrates that there is a frequency and field-strength-dependent growth modification of *Bacillus subtilis*, FJ7 mutant bacteria, and that macrofiber right-handed helix formation can be altered when exposed to the magnetic field. Further studies will be needed to map the entire range of frequencies where the growth rate can be optimized, and studies will be needed at the cellular level to examine the causes of the major modification of the macrofiber orientation.

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