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Change in broth culture is associated with significant suppression of *Escherichia coli* death under high magnetic field

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Abstract

When *Escherichia coli* B was cultivated under an inhomogeneous magnetic field of 5.2–6.1 T, a significant 100,000-fold suppression of cell death was observed [Bioelectrochemistry 53 (2001) 149]. The limited magnetic field exposure for 12 h after logarithmic growth phase was sufficient to observe similar suppressive effects on cell death [Bioelectrochemistry 54 (2001) 101]. These results suggest some possible changes in either the medium or the cells during the magnetic field exposure. When the cell-free filtrate of the broth cultured under the magnetic field for 10 h and the cells of *E. coli* cultivated under the geomagnetic field for 30 h were mixed, and the mixture was subsequently cultivated under the geomagnetic field, the number of cells observed in the filtrate exposed to the high magnetic field was 20,000 times higher than that in the filtrate exposed to the geomagnetic field. When the cells cultivated under the magnetic field for 10 h and the cell-free filtrate of the broth culture exposed to the geomagnetic field were mixed, only a 50-fold difference in the number of cell between under the magnetic field and under the geomagnetic field was observed. This suggests that the filtrate of the broth culture exposed to the magnetic field is primarily responsible for the cell death suppression. It was also revealed that the small difference in pH of the filtrates of the broth culture between under the magnetic field and under the geomagnetic field was critical for the cell death suppression.

Keywords: Broth culture; *Escherichia coli*; High magnetic field

1. Introduction

We have previously studied the magnetic field effect on biological reactions using the superconducting magnet bio-system (SBS), and the suppression of death of *Escherichia coli* cells [1–4], the inhibition of the sporulation of *Bacillus subtilis* cells [5], and the absence of a significant effect on mammalian cells [6] were reported.

The drastic 100,000-fold suppression of *E. coli* cell death was observed in one-fourth concentration of the Luria-Bertani (LB) medium supplemented with 1.5 g/l glutamic acid under an inhomogeneous 5.2–6.1 T magnetic field [1]. This suppressive effect was observed when the magnetic field exposure time was restricted to 12 h after the logarithmic growth phase [2]. These data suggest that the factor(s) suppressing cell death emerged during the 12-h magnetic field exposure. In this study, we separated the cell-free filtrate of the broth culture and cells of *E. coli* and the contribution of the two factors on the magnetic field effect was investigated. The effects of accelerating water vaporization under a magnetic field and change in pH on cell death suppression were also analyzed.

2. Materials and methods

2.1. Bacterial strain and media used

E. coli B (wild-type strain), which does not exhibit an auxotrophic genotype, was used throughout the experiments. A Luria-Bertani (LB) medium containing 10 g of polypepton (Nihon Pharmaceutical, Tokyo), 5 g of yeast extract, and 5 g of NaCl (per liter of distilled water; pH 7) was used in the preculture of *E. coli*. The LB/4 + 1.5 E medium which contains one-fourth concentration of the LB medium supplemented with 1.5 g/l glutamic acid was used in the main culture because significant suppression of cell

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death of *E. coli* under an inhomogeneous magnetic field was observed in this LB/4 + 1.5 E medium [1]. A LB agar medium, in which 1.5% agar was added to the LB medium, was used to count the viable cells at 37 °C which was expressed in colony-forming units (CFU).

2.2. Suppression of cell death in supernatant of broth culture prepared after exposure to magnetic field

E. coli B was cultivated in a LB medium overnight in a shaking unit at 43 °C, and 0.05 ml of the broth culture was inoculated into 5 ml of a fresh LB/4 + 1.5 E medium and cultured for about 2 h in order to obtain cells in the logarithmic growth phase. This broth culture was inoculated into the fresh LB/4 + 1.5 E medium at an initial cell concentration of 10^2–10^3 cells/ml, and the mixture was cultivated at 43 °C for 20 h under a geomagnetic field. Then, 15 ml of this broth culture was transferred into two identical conical flasks (nominal volume, 50 ml) and one flask was exposed to a high magnetic field and the other to a control geomagnetic field. These flasks were further shaken at 120 strokes per minute (spm) at a controlled temperature of 43 °C for 10 h. This 10-h cultivation period corresponds to the shaded area shown in Fig. 1. The broth cultures under the high magnetic field and the geomagnetic field were centrifuged at 6000 × *g* for 5 min, and the supernatant of each culture was obtained. Separately, the cells were recovered from the 10-ml broth culture grown in the LB/4 + 1.5 E medium at 43 °C for 30 h under geomagnetic field and were suspended in each of the recovered supernatants in the same flask described above. The flasks were shaken at 43 °C under a geomagnetic field. From the start of this cultivation, the cultivation time was defined as 0 h, and the change in the number of cells was determined periodically.

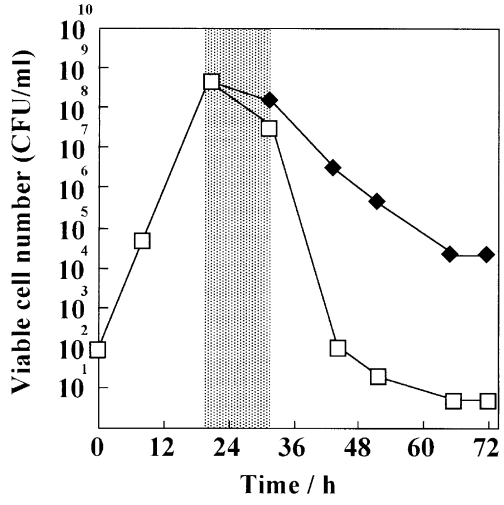

Fig. 1. Change in number of viable cells of *E. coli* B grown under the inhomogeneous magnetic field of 5.2–6.1 T (◆) and under the geomagnetic field as control (□). Shaded area indicates the period when magnetic field was applied: 20–32 h.

2.3. Suppression of cell death by cells obtained from broth culture exposed to high magnetic field

The cells of *E. coli* B were cultivated for 20 h under the geomagnetic field by the same method described in Section 2.2, and the following 10-h cultivation was carried out under either a high magnetic field of 5.2–6.1 T or a geomagnetic field. Cells of a 10-ml broth culture exposed to the magnetic field or to the geomagnetic field were recovered by centrifugation, and the cells recovered under each exposure condition were suspended in 10-ml supernatant in a flask prepared from the broth culture of the LB/4 + 1.5 E medium at 43 °C which was cultivated for 30 h under the geomagnetic field. These flasks were further cultivated under the geomagnetic field and the change in the number of cells was determined periodically from the start of this cultivation.

2.4. Counting of viable cells

Viable cells were counted as follows: the periodically sampled broth culture was diluted appropriately in 0.05 M phosphate buffer (pH7) and was spread on the LB agar medium following a 12-h incubation at 37 °C. The colonies that appeared on the plates were counted and expressed in colony-forming units (CFU).

2.5. Structure of superconducting magnet biosystem (SBS)

As the features of the SBS were described in detail in a previous paper [7], specifications important in this experiment are described here. SBS is composed of three parts, namely, a superconducting magnet, a temperature control unit and a shaking unit. The superconducting magnet has a 16-cm (diameter) bore in the horizontal direction which can produce a magnetic field strength of 0.5–7 T. The area ± 10 cm from the center of the magnet is the homogeneous magnetic field region, outside of which the strength of the magnetic field gradually decreases with a maximum gradient of 23 T/m. If bacterial cultivation is operated in this area with a magnetic field gradient, the cultivation can be exposed to an inhomogeneous (time-varying) magnetic field. In this experiment, an inhomogeneous magnetic field of 5.2–6.1 T was used. A control experiment without exposure to the high magnetic field was carried out under the geomagnetic field, which was covered with a double-walled cylindrical shell of Permalloy to shield the culture from the high magnetic field. The flasks containing the broth culture were shaken horizontally at 120 spm with an amplitude of shaking width of ± 2 cm to completely mix the culture and supply sufficient oxygen. The temperature difference between the two incubators as detected by thermometers was fed back to the temperature control unit

so as to regulate the temperature difference within ± 0.1 °C using a computer.

3. Results

3.1. Cell death suppression in supernatant prepared from broth culture exposed to high magnetic field

Fig. 1 shows that magnetic field exposure for a limited time of 12 h after the end of the logarithmic growth phase suppressed cell death by 100,000-fold at 48 h compared with the control.

Since the cell death suppression continued even after the cessation of the magnetic field exposure, we speculated that significant change may have occurred in either the cells or the medium during the magnetic field exposure. First, the supernatant from the broth culture cultivated under the magnetic field was used for further analysis.

The supernatant from the broth culture that was exposed to the inhomogeneous magnetic field of 5.2–6.1 T for 10 h after the end of the exponential growth phase or that from the control broth culture under the geomagnetic field was prepared. Then, the cells harvested from the broth culture grown under the geomagnetic field were suspended in the two supernatants, and incubation at 43 °C was continued. Fig. 2 shows the change in the number of viable cells. The number of viable cells was about 20,000 times higher than that detected in the cell suspension prepared from the high magnetic-field-exposed broth culture compared to the geomagnetic-field-exposed control. The same experiment was repeated four times. The initial pH values at the start of the cultivation in Fig. 2 were measured to be 9.35 ± 0.01 for the cell suspension exposed to the high magnetic field, and 9.42 ± 0.02 for that exposed to the geomagnetic field after four independent experiments. A difference of 0.07 in the two pH values was significantly large compared with the experimental error.

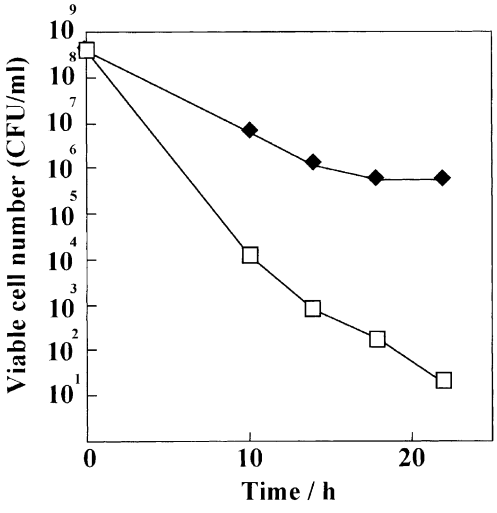

Fig. 2. Change in number of viable cells of *E. coli* B under the geomagnetic field when the cells grown under the geomagnetic field were suspended in the supernatant of the culture grown under the inhomogeneous magnetic field of 5.2–6.1 T (◆) or in the supernatant of the culture grown under the geomagnetic field (□). Initial pH values of the supernatant: magnetic field, 9.35 ± 0.01 (average ± SD, $n = 4$); geomagnetic field, 9.42 ± 0.02 (average ± SD, $n = 4$). SD: standard deviation, n: number of experiments.

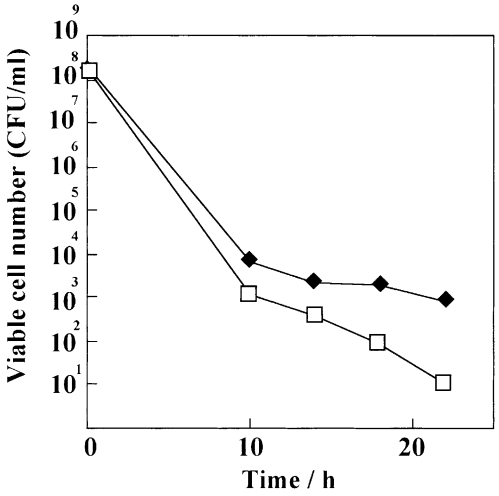

Fig. 3. Change in number of viable cells of *E. coli* B grown under the geomagnetic field when the cells recovered from culture grown under the inhomogeneous magnetic field of 5.2–6.1 T (◆) and the cells grown under the geomagnetic field (□) were suspended in the supernatant of the culture grown under the geomagnetic field. Initial pH values of the supernatant: magnetic field, 9.35 ± 0.03 (average ± SD, $n = 4$); geomagnetic field, 9.35 ± 0.04 (average ± SD, $n = 4$). SD: standard deviation, n: number of experiments.

3.2. Cell death suppression by cells prepared from broth culture cultivated under high magnetic field

The supernatant prepared from the broth culture cultivated under the high magnetic field showed to be a primary factor for the suppression of cell death as described in Section 3.1. Then, the cells grown under the high magnetic field were investigated with respect to the suppression of cell death. The cells recovered from the broth culture incubated under the high magnetic field and under the geomagnetic field were suspended in the supernatant prepared from the broth culture grown under the geomagnetic field culture, and the incubation was started. As shown in Fig. 3, 22 h after the start of cultivation, about 50 times higher number of cells was detected in the culture of cells grown under the magnetic field compared with that of the cells grown under the geomagnetic field. This suppressive effect on the cell death, however, was significantly smaller than that observed in the supernatant, in which about 20,000 times higher number of viable cells was observed. The initial pH values of the two cell suspensions at the start of the cultivation for the four independent experiments were 9.35 ± 0.03 and 9.35 ± 0.04. The differences in pH values among the four experiments were almost identical.

3.3. Effect of the water evaporation from the culture broth on the suppression of the cell death

Since the acceleration of water vaporization under a high magnetic field was reported [8], water vaporization from the LB medium under the magnetic field as well as the geomagnetic field was measured, the results of which are as shown in Fig. 4 where the LB media were incubated at 43 °C at the shaking speed of 120 spm. The rate of water vaporization was determined as follows: each flask containing the LB medium was weighed periodically and the reduction in weight was divided by the initial value and was expressed in % (w/w). After 12-h incubation, the water vaporization rate of the control was about 3%, whereas that under the magnetic field was about 10%; thus a clear acceleration in vaporization under the magnetic field was observed. The vaporization of water may have an effect on the degree of cell death suppression. To examine this possibility, the experiment described in Section 3.1 was modified as follows: the difference in the amount of vaporized water after 10-h incubation between magnetic-field- and geomagnetic-field-exposed cultures was about 1 g, thus distilled water was added to the flask under the magnetic field to compensate for this water loss at the initial period of cultivation. The weight of the water added was precisely determined using an electronic balance. Then, the same experiment as that in Section 3.1 was conducted. The number of viable cell under the high magnetic field was 15,000 times higher than that under the geomagnetic field as shown in Fig. 5.

3.4. Effect of pH on suppression of cell death

When the significant suppression on cell death under the high magnetic field was observed as shown in Fig. 2, the pH value of the broth culture showed only 0.07 difference between the magnetic field exposed and the control cultures after 10-h cultivation. To investigate the effect of pH on cell death suppression, the initial pH of the supernatant obtained from the culture cultivated under the magnetic field was adjusted to that cultivated under the geomagnetic field by

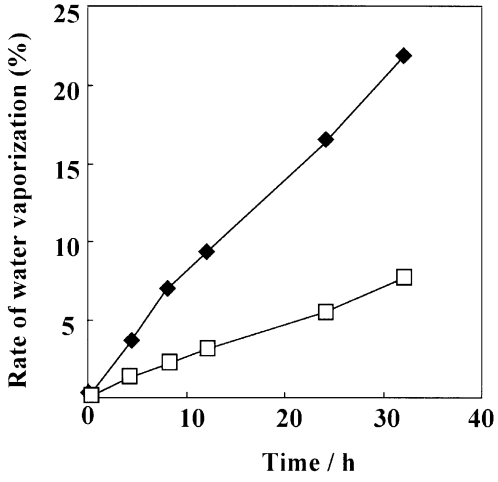

Fig. 4. Change in the ratio of water vaporization in the LB medium incubated under the inhomogeneous magnetic field of 5.2–6.1 T (◆) and under geomagnetic field (□).

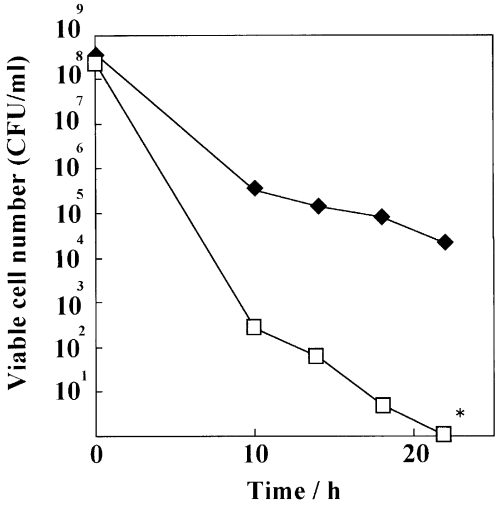

Fig. 5. Change in number of viable cell of *E. coli* B grown under the geomagnetic field when the cells grown under the geomagnetic field were suspended in the supernatant of the culture grown under the inhomogeneous magnetic field of 5.2–6.1 T (◆) or in supernatant of the culture grown under the geomagnetic field (□). Cells recovered from the geomagnetic-field-exposed culture were suspended in both supernatants. At the start of incubation, about 1 g of distilled water was added to compensate for the amount of evaporated water. *: lower than the detection limit.

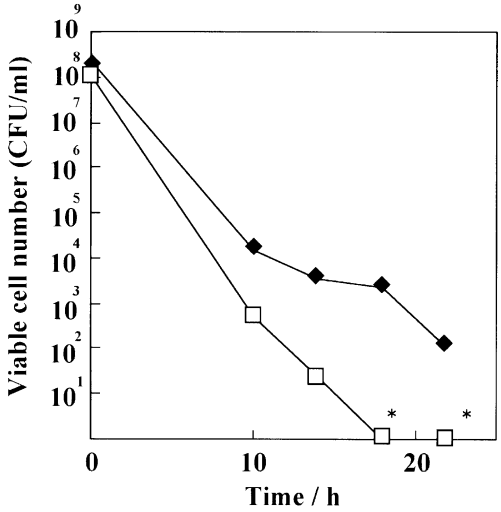

Fig. 6. Change in number of viable cells of *E. coli* B grown under the geomagnetic field when the cells grown under the geomagnetic field were suspended in the supernatant of the culture grown under the inhomogeneous magnetic field of 5.2–6.1 T (◆) or in the supernatant of the culture grown under the geomagnetic field (□). At the start of incubation, the pH of the supernatant from the culture grown under the magnetic field was adjusted to that of the control by adding 50 mM NaOH solution, where a pH difference of 0.07 ± 0.01 was detected in the experiment described in Section 3.1. *: lower than the detection limit.

adding 50 mM NaOH, and the same experiment as that described in Section 3.1 was conducted.

The number of viable cells in the supernatant prepared from the magnetic-field-exposed culture was about 200 times higher than that prepared from the geomagnetic-field-exposed culture (control) after 12-h incubation as shown in Fig. 6. This showed a significant increase in the number of dead cells by only the adjustment of the initial pH values.

4. Discussion

Factors leading to the suppression of death of *E. coli* cells were found both in the supernatant and the cells from the broth culture grown under the magnetic field. In particular, for the supernatant from the broth culture grown under the magnetic field, about 20,000 times more cells survived than that for the control. This suppressive effect on cell death was almost the same as that observed in the experiment, in which the broth culture was exposed to the magnetic field for 12 h after the end of the logarithmic growth phase as shown in Fig. 1. On the other hand, the cells prepared from the culture grown under the high magnetic field showed about 50 times higher number of viable cells which was significantly lower than that 20,000 times higher in the case of the supernatant. From these results, the suppressive effect on cell death after the cessation of the magnetic field exposure was thought to be mainly due to the change in the medium during the 10-h magnetic field exposure after the exponential growth phase.

When the inhomogeneous magnetic field was applied to the medium during incubation, evaporation of water from the medium was enhanced leading to the concentration of the medium. This concentration of the medium may have an effect on cell death suppression. This effect was tested by adding distilled water to the medium to compensate for the water lost through vaporization. The number of cells determined under the high magnetic field in this experiment was about 15,000 times higher than that of the control. Compared with about 20,000 times higher number of cells observed in Fig. 1, the effect of water evaporation on cell death suppression was assumed to be insignificant.

When a significantly large magnetic field effect was observed, a slight pH difference between the broth culture under the high magnetic field and that under the control geomagnetic field was observed. The high magnetic field effect was reduced by the two orders of magnitude when the pH values of the two media were adjusted. The reduction in the degree of suppression by two orders of magnitude indicates that the main factors causing the significant cell death suppression are associated with the change in pH of the medium.

The pH changes in the culture are caused by several factors. One of them is inferred as the metabolic change in the cells. Amino acids in the LB medium were converted to alkaline products through their metabolism [9]. In particular, glutamic acid, which was one of the main components of the medium, is reported to produce ammonia and basic amino acids such as arginine, which are produced from ornithine and citrulline [10]. These metabolites shifted the medium's pH to the alkaline region. During the period of the decrease in the number of viable cells, the pH of the medium was in the alkaline region. If the amino acid metabolic pathways that cause the pH change to alkaline were affected by the high magnetic field, the pH of the broth culture under the high magnetic field would be lower than that of the broth culture under the geomagnetic field.

Another possible explanation for the lower pH of the medium under the high magnetic field is the physico-chemical effect of the magnetic field on the supernatant of the broth culture. As a high magnetic field under 10 T was reported to enhance the dissolution of carbon dioxide (CO_2) by 27% in the presence of oxygen [11], the H^+ ions generated through the equilibrium reaction of $CO_2 + H_2O \leftrightarrows HCO_3^- + H^+$ are expected to increase [H^+] that will result in lower pH. According to the equilibrium constant of CO_2 pKa 6.51 at 30 °C in water and 27% increase in solubilized CO_2 concentration, the decrease in pH was calculated to be about 0.1.

The movement of water molecules to the lower magnetic field under the high magnetic field was reported [12]. During vigorous shaking in this study, the contribution of the movement of water molecules is not clear, but it is presumed that the increase in the water surface area exposed to the magnetic field due to this effect may enhance the solubilization of CO_2 or vaporization of ammonia, if any, which will reduce the pH of the medium.

Even after the pH of the supernatant from the broth culture exposed to the magnetic field was adjusted to that of the control, about 200 times higher number of cells was detected. This suggests the existence of other factors involved in the full suppression effect of the magnetic field on cell death. For further analysis on the change in metabolism, a DNA array analysis or protein identification as well as a chemical analysis of the medium will be necessary.

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