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Far-infrared irradiation-induced injuries to *Escherichia coli* at below the lethal temperature

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***Escherichia coli* in phosphate-buffered saline irradiated with far-infrared (FIR) energy was injured and killed even under the condition where the bulk temperature of the suspension was maintained below the lethal temperature. Using four kinds of antibiotics (penicillin G, chloramphenicol, nalidixic acid and rifampicin), we investigated the FIR irradiation-induced damage to *E. coli* on the basis of the sensitivity changes to the antibiotics. FIR irradiation increased the organism's sensitivity to rifampicin both below and above the lethal temperature. The increase in sensitivity to chloramphenicol was observed only when FIR irradiation occurred above the lethal temperature. These results suggest that the mechanism of FIR irradiation-induced death in *E. coli* differs according to whether the radiation exposure occurs above or below the lethal temperature.** *Journal of Industrial Microbiology & Biotechnology* (2000) **24,** 19–24.

Keywords: infrared radiation; pasteurization; antibiotic; *Escherichia coli*; injured cell; lethal temperature

Introduction

The importance of preventing microbial contamination of foods and food preparation areas is becoming increasingly clear. The use of infrared (IR) heating for thermal decontamination of working environments offers the advantage that no heat-transfer medium is needed. Far infrared (FIR, about 3–1000 μm wavelength) radiation is easily absorbed by water and organic materials, which are the main components of food, and thus offers considerable potential for efficient pasteurization.

We have studied the use of FIR for pasteurization [4–7,15–17]. Compared with thermal conductive heating, FIR irradiation was more effective in pasteurizing vegetative bacterial cells [4,17]. Furthermore, FIR irradiation caused heat activation and death of *Bacillus subtilis* spores over a temperature range in which thermal conductive heating had no effect on spore viability [16]. The pasteurization effects of FIR reportedly were due both to the absorption of radiative energy by the bacterial suspension in a very thin volume near the surface and to an increase in the bulk temperature of the suspension [4,6]. Furthermore, estimations of the temperature distribution within the FIR-irradiated microbial suspension suggest that the temperature of the surface region is significantly higher than the bulk temperature [5,7].

How bacteria in suspensions irradiated by FIR at sublethal temperatures are damaged remains unclear. In previous work [13–15], FIR irradiation-induced bacterial damage was evaluated by assessing changes in the susceptibility to antibiotics. In this work, damage to *Escherichia coli* irradiated by FIR below and above the lethal temperatures was compared using antibiotic sensitivity. In addition, estimations of the temperature distribution within bacterial cells in relation to the changes in extracellular temperature were used to analyze the mechanism of FIR pasteurization.

Materials and methods

Cell culture

Escherichia coli 745 was obtained from the Tokyo Metropolitan Research Laboratory of Public Health. The bacteria were stored at –80°C, then thawed and incubated in Brain Heart Infusion broth (Difco, MI, USA) at 37°C for 24 h. The cells were washed once in 0.05 M phosphate-buffered saline (PBS, pH 7.0) and resuspended in the PBS at a final concentration of approximately 10^6 colony forming units (CFU) ml^{-1}. The bacterial suspension was kept in iced water before it was used in experiments. The number of viable CFU was determined by pour-plating on Sensitivity Desk Agar-N (Nissui Seiyaku, Tokyo, Japan) without any agents (non-selective medium).

FIR irradiation

The FIR irradiation apparatus and the pasteurization sample were as described previously [5]. In essence, the irradiation chamber is made of aluminum plates, and a mullite cylinder FIR heater (300 × 15 mm) with a reflector was placed at the top. Bacterial suspension (7.5 ml) in a stainless steel petri dish (14.5 × 88.5 mm) was irradiated at a power (q_{ir}) of 7.57 kW m^{-2}. Figure 1 shows the spectral distribution of irradiated power to the bacterial suspension at 7.57 kW m^{-2}. During irradiation, the suspension was agitated by a rotary

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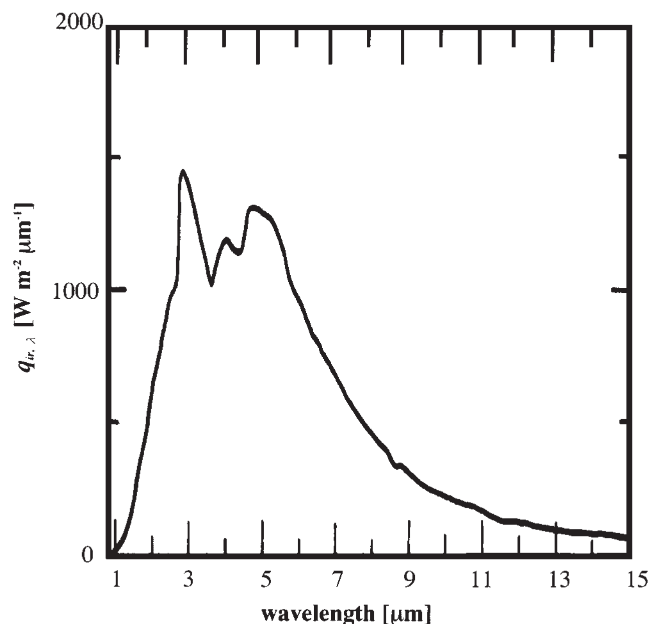

Figure 1 Spectral distribution of radiative power delivered to the bacterial suspension (7.57 kW m^{-2}).

shaker at 180 rpm and cooled from the bottom of the petri dish to keep the bulk temperature of the suspension below the lethal temperature. After irradiation, the dish was cooled rapidly by placing it in iced water.

For measurement of the suspension temperature, CA-thermocouples sheathed by a stainless steel tube (0.25 mm ∅) were placed horizontally at positions close to the surface, the center and the bottom of the suspension. The temperatures at these three points were recorded during irradiation. The difference in temperature measured was within 0.2°C, the average values are presented in Figure 2, showing that the bulk temperature did not rise higher than 40°C. The lethal temperature of *E. coli* used here is about 55°C. Therefore, the bulk temperature of the suspension was kept below the lethal temperature of the *E. coli*.

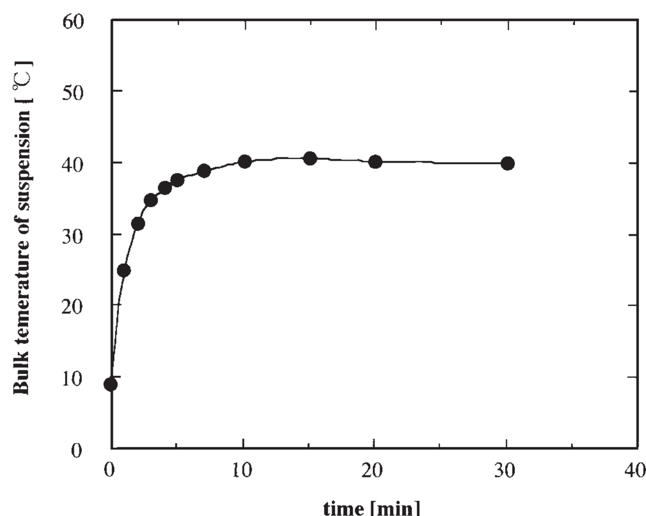

Figure 2 Transient behavior of bulk temperature of a bacterial suspension irradiated by FIR. $q_{ir} = 7.57$ kW m^{-2}; $V_l/S_h = 0.91$ mm.

The irradiation power (q_{ir}) that the bacterial suspension is exposed to was measured using previously described methods [8], q_{ir} was estimated by determining the evaporation rate of the water layer irradiated by the FIR heater.

Conductive heating

The same conductive heating apparatus was used as previously reported [4]. The bacterial suspension (1.5 ml) was heated in a test tube (10 mm × 100 mm) in a water bath. The tube was removed after 5 min and cooled in iced water.

Examination of changes in antibiotic sensitivities

After irradiation or conductive heating, the cooled suspension was diluted with 0.85% (w/v) saline solution. The diluted suspension was pour-plated with agar medium. Duplicate plates were prepared for each dilution. Sensitivity Disk Agar-N was used as a non-selective medium. Agar media containing the antibiotics shown in Table 1 was used to analyze FIR-induced microbial damage. The required concentration of each antibiotic in the agar medium had already been determined previously [15]. After 48 h incubation at 37°C, colonies were counted using an automated colony-counter (Toyo CA-9, Yokohama, Japan). The damage to the bacteria was assessed on the basis of changes in bacterial antibiotic sensitivity using procedures described previously [15].

Results and discussion

Sensitivity change of E. coli irradiated by FIR below the lethal temperature

FIR irradiation increased the temperature of the bacterial suspension so it was cooled to maintain the bulk temperature at 40°C, considerably below the lethal temperature for *E. coli* (Figure 2). The pasteurization effect achieved by FIR irradiation is shown in Figure 3. The ordinate is the ratio of the number of *E. coli* CFU post-irradiation (N_1) to the number of non-irradiated CFU (N_{10}) and represents the survival ratio. The value of N_1/N_{10} in Figure 3 is the survival ratio of *E. coli* on the non-selective medium. V_l/S_h is the ratio of the volume of the suspension to the area irradiated by FIR and is equivalent to the depth of the suspension. The survival ratio of *E. coli* decreased with an increase in irradiation. As most of the suspension was maintained at 40°C, the pasteurization effect by FIR irradiation can not be attributed to an elevation in the bulk temperature.

The damage induced in *E. coli* by FIR irradiation below the lethal temperature was assessed by measuring bacterial antibiotic sensitivity (Figure 4). The abscissa, C/C_{MAX}, rep-

Table 1 Primary inhibitory action of selective antibiotics

Reagent	Primary inhibitory action
Penicillin G (PCG)[a]	synthesis of cell wall
Chloramphenicol (CP)[a]	synthesis of protein (ribosome)
Nalidixic acid (NA)[b]	synthesis of DNA
Rifampicin (RFP)[b]	synthesis of RNA (RNA polymerase)

[a]Sigma Chemical Company (St Louis, MO, USA).
[b]Daiichi Pharmaceutical Co Ltd (Tokyo, Japan).

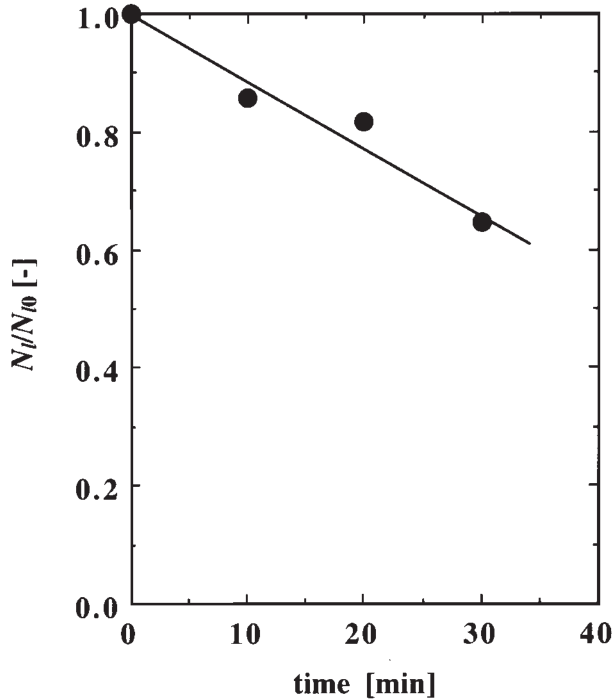

Figure 3 Pasteurization of *E. coli* by FIR irradiation below the lethal temperature (40°C). $q_{ir} = 7.57$ kW m^{-2}; $V_l/S_h = 0.91$ mm.

resents the ratio of a specified concentration (C) to the maximum concentration of the antibiotic in the agar medium (C_{MAX}). The upper limit of the concentration of antibiotic in the medium (C_{MAX}) was defined as the maximum concentration at which the antibiotic exhibited no effect on colony formation by non-irradiated *E. coli*. The C_{MAX} of each of the four antibiotics employed was determined previously [15]. The incubation on agar media containing antibiotics at C_{MAX} allowed detection of any slight damage to the bacterial cells. After 10 min of irradiation, the value of the survival ratio on the non-selective medium decreased to approximately 0.9. In addition, the survival ratio on rifampicin (RFP) medium declined with C/C_{MAX} (Figure 4a), demonstrating that *E. coli* had enhanced sensitivity to RFP. Increased irradiation time resulted in increased sensitivity to RFP (Figure 4b and c). On the other hand, conductive heating at 40°C neither reduced the survival ratio nor changed the antibiotic sensitivities of *E. coli* (data not shown). We therefore concluded that *E. coli* was not adversely affected by conductive heating at 40°C.

Undamaged bacteria are able to grow on both the non-selective medium and the antibiotic-containing media. However, a proportion of damaged microbial cells can form colonies on non-selective medium, but not on the medium containing antibiotics [9]. The difference in colony formation in the presence and absence of antibiotic can thus be used as a measure of injury. Table 1 shows the primary actions of the antibiotics used in these experiments. As they each target specific aspects of cellular mechanism, antibiotic sensitivity is a simple means of detecting and classifying the damage induced by physical and chemical stresses, as a given stress induces different changes in microbial antibiotic sensitivity [13–15]. RFP acts on RNA polymerase to inhibit initiation of DNA-dependent RNA synthesis [3,19,21]. The data presented here suggest that FIR irradiation below the lethal temperature caused damage in *E. coli*, that is reflected in the change in sensitivity to RFP.

In previous work [15], the bacterial sensitivity change caused by FIR irradiation above the lethal temperature was investigated. Figure 5 shows the transient behavior of the bacterial suspension irradiated by FIR without cooling. The irradiated power was 3.2 kW m^{-2}. For a comparison with FIR irradiation above the lethal temperature, the following equation is defined:

$$R_{IN} = \frac{N_l(C/C_{MAX} = 0) - N_l(C/C_{MAX})}{N_l(C/C_{MAX} = 0)} = 1 - \frac{N_l(C/C_{MAX})}{N_l(C/C_{MAX} = 0)} \quad (1)$$

R_{IN} is the ratio of the damage cells to that of viable cells. As the value of R_{IN} for an antibiotic approaches unity, the injury becomes more serious.

Figure 6 illustrates the differences in antibiotic sensi-

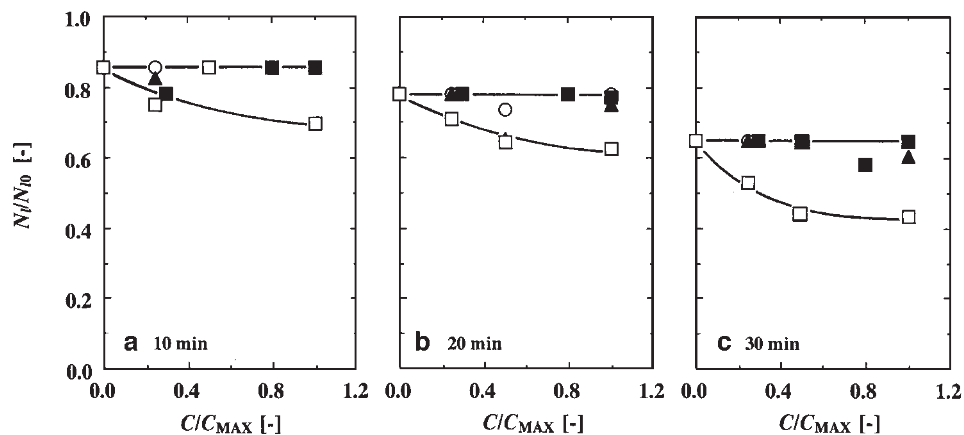

Figure 4 Changes in antibiotic sensitivities of *E. coli* by FIR irradiation below the lethal temperature: (a) 10 min, (b) 20 min, (c) 40 min: $q_{ir} = 7.57$ kW m^{-2}; $V_l/S_h = 0.91$ mm. ○, Penicillin G; ▲, chloramphenicol; ■, nalidixic acid; □, rifampicin.

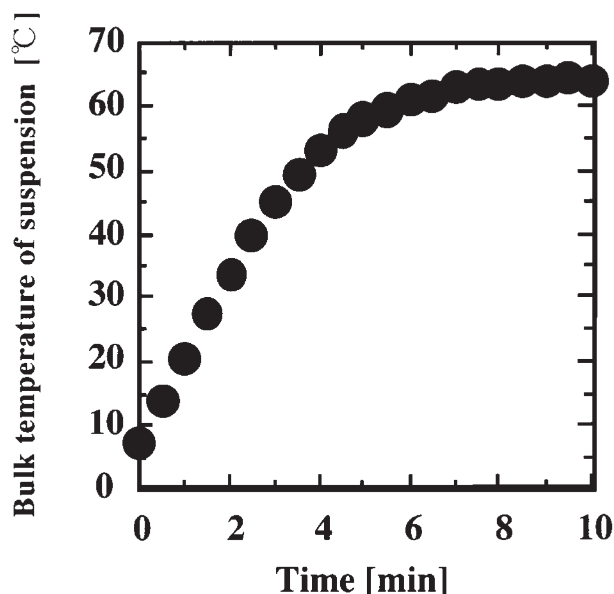

Figure 5 Transient behavior of the bulk temperature of a bacterial suspension irradiated by FIR at supralethal temperature. $q_{ir} = 3.22$ kW m^{-2}; $V_l/S_h = 3.3$ mm.

tivities between bacteria irradiated with FIR above and below the lethal temperature. The R_{IN} values in the case of FIR irradiation above the lethal temperature for 7 min were calculated from results obtained previously [15]. After irradiation for 7 min, the bulk temperature of the suspension was 61°C, and the survival ratio on the non-selective medium ($C/C_{MAX} = 0$) was approximately 0.3. As shown in Figure 6a, FIR irradiation above the lethal temperature increased the sensitivity of *E. coli* to both chloramphenicol (CP) and RFP. CP acts on ribosomes and inhibits protein synthesis [2,10–12]. *E. coli* irradiated by FIR above the lethal temperature will receive more serious injuries. Below the lethal temperature, viability was reduced with no change in CP sensitivity, only RFP-treated cells being affected (Figure 6b). Thus, the mechanism of pasteurization by FIR irradiation below the lethal temperature appears to differ from that above the lethal temperature.

We were particularly interested in the relationship between the increase in sensitivity to CP and the rise in the temperature of the suspension above the lethal temperature. When *E. coli* cells were heated for 5 min by thermal conduction at different temperatures and subsequently incubated with the non-selective medium, the viability declined sharply at approximately 55°C (Table 2). The variation of the colony counts on the medium containing CP at the C_{MAX} of 2.0 μg ml^{-1} was very similar to that on the non-selective medium until supralethal temperatures were reached. The change in the sensitivity to CP might provide an indication of whether or not the internal temperature of a bacterial cell reaches the 'lethal' temperature during heat treatment. However, as the bulk temperature of the suspension was maintained below the lethal temperature, it seems unlikely that the internal temperature of circulating bacteria would gradually increase above the lethal temperature. It is probable that the internal temperature of a given bacterial cell would remain marginally below the lethal temperature

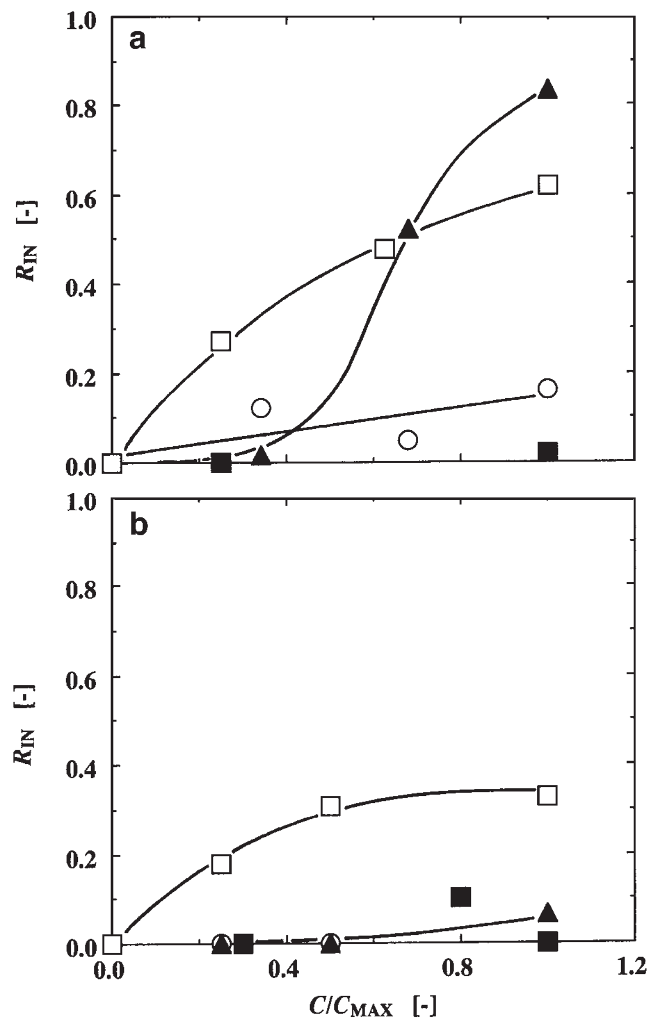

Figure 6 Changes in antibiotic sensitivities of *E. coli* by FIR irradiation at sub- and supralethal temperature: (a) FIR irradiation at supralethal temperature at 3.22 kW m^{-2} for 7 min; (b) FIR irradiation below the lethal temperature at 7.57 kW m^{-2} for 40 min. ○, Penicillin G; ▲, chloramphenicol; ■, nalidixic acid; □, rifampicin.

even during FIR irradiation, so we analyzed the temperature fluctuations in more detail.

Temperature change in a bacterial cell in a suspension irradiated by FIR

After previous study [5], in which the temperature distribution of the suspension irradiated by FIR was determined, it was suggested that the temperature in a thin surface region was higher than the temperature of the bulk suspension. The temperature in the surface region cannot be measured experimentally because the thickness of the affected portion is calculated to be some 10 μm. When the irradiated power was 7.57 kW m^{-2}, the estimated surface temperatures of the suspension rose to 53.1°C for 0.1 s, 80.3°C for 0.3 s and over 100°C for 0.5 s, even when the bulk temperature of the suspension was maintained at 40°C [5].

Agitation of the bacterial suspension should give a uniform temperature distribution. Despite this, as the surface region will have a higher temperature due to FIR irradiation (Figure 7), the internal temperature of bacterial cells will

Table 2 Changes in viable counts of *E. coli* heated by thermal conduction for 5 min at different temperatures and their sensitivity to chloramphenicol (CP)

Concentration of CP	Viable cells (CFU ml^{-1})					
	Initial	0°C	40°C	50°C	55°C	60°C
0 μg ml^{-1} (non-selective medium)	5.12×10^6	5.04×10^6	5.15×10^6	5.02×10^6	4.63×10^6	4.46×10^4
2.0 μg ml^{-1} (C_{MAX})	5.12×10^6	5.06×10^6	5.18×10^6	4.94×10^6	4.18×10^6	2.21×10^4

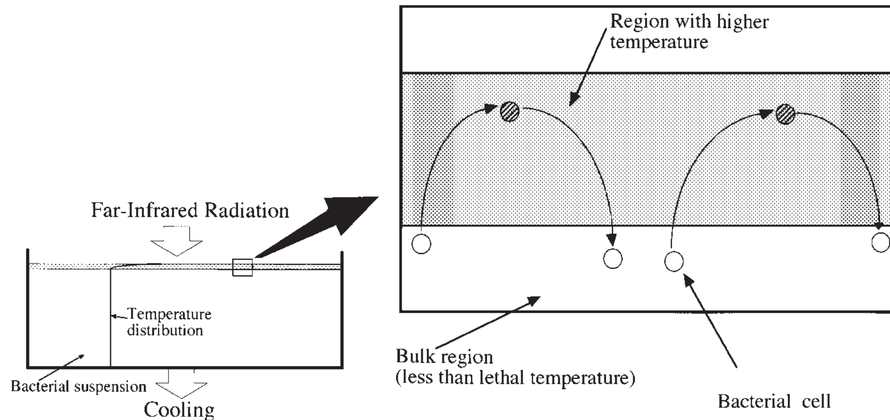

Figure 7 Model of bacterial suspension irradiated by FIR below its lethal temperature.

vary with the extracellular temperature. For this study, we modeled the process by considering unsteady-state heat conduction in a spherical solid. We assume a bacterial cell to be equivalent to a homogeneous spherical solid of radius R with thermal properties equivalent to water, the main component of the cell. Radiation energy absorbed by bacteria in suspension is not discussed. Consider that a bacterial cell, initially at a uniform temperature T_{CO}, suddenly enters a region of higher or lower temperature T_1 at $t > 0$. Using the following dimensionless variables:

$$\Theta_T = \frac{T_1 - T_C}{T_1 - T_{C0}} \qquad (2)$$

$$\xi = r/R \qquad (3)$$

$$\tau = \alpha t/R^2 \qquad (4)$$

in which T_C and α are the temperature of a bacterial cell and the thermal diffusivity of water, respectively, the differential equation describing the boundary conditions is:

$$\frac{\partial\Theta_T}{\partial\tau} = \frac{1}{\xi^2}\frac{\partial}{\partial\xi}\left(\xi^2\frac{\partial\Theta_T}{\partial\xi}\right) \qquad (5)$$

$$\text{I.C. } \tau = 0,\ 0 \leqq \xi \leqq 1;\ \Theta_T = 0 \qquad (6)$$

$$\text{B.C.1}: \tau > 0,\ \xi = 1;\ \Theta_T = 1 \qquad (7)$$

$$\text{B.C.2}: \quad \xi = 0;\ \Theta_T = \text{finite} \qquad (8)$$

Finally, the temperature distribution in the spherical solid as a bacterial cell is given by the following equation [18]:

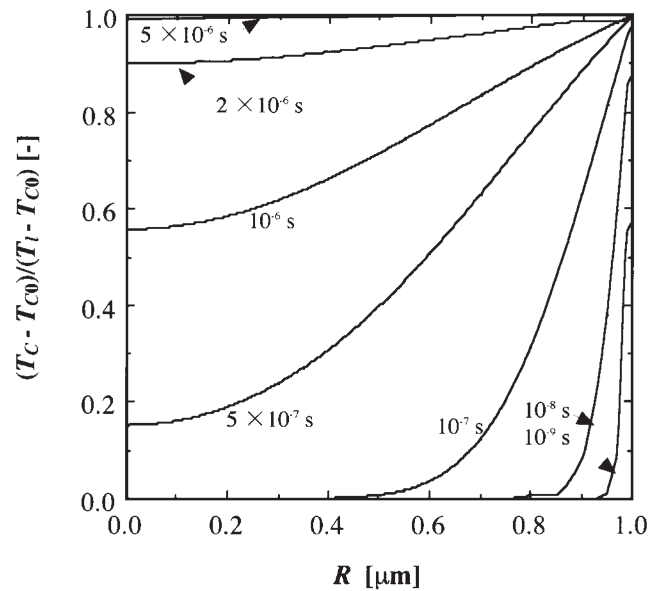

Figure 8 Changes in internal temperature (T_C) of a bacterial cell for the case in which the cell with radius R, initially at a uniform temperature T_{CO}, suddenly enters a region with higher temperature T_1.

$$\frac{T_C - T_{C0}}{T_1 - T_{C0}} = 1 + \frac{2R}{\pi r}\sum_{n=1}^{\infty}\frac{(-1)^n}{n}\sin\left(\frac{n\pi r}{R}\right)e^{-}\frac{(n\pi)^2\alpha_t}{R^2} \qquad (9)$$

Here, assuming that the radius (R) of a bacterial cell is 1 μm, the predicted transient behavior of the temperature distribution inside a cell is shown in Figure 8.

Bacterial cells readily respond to changes in the external temperature. When a bacterial cell enters the surface region with its higher temperature, the internal temperature of the cell becomes equal to the external temperature for a period for 5×10^{-6} s. In the present experiments, it is very difficult to establish how long the bacteria stay in the region of higher temperature due to agitation of the rotary shaker. However, we may approximate the time by considering that the bacterial suspension is agitated at 180 rpm, and therefore the time of one agitation cycle is about 0.33 s. In this study, the depth of the bacterial suspension was approximately 1 mm. If a bacterial cell moves back and forth between the surface and the bottom of the suspension in 0.33 s, it will take about 1.7×10^{-4} s for the cell to move a distance of 1 μm. This period is very short, but is sufficient for the internal temperature of the cell to rise to the external temperature, especially when we consider the depth of the surface region to be about 10 μm. When the cell leaves the region of higher temperature, the intercellular temperature rapidly falls to that of the bulk temperature. The results shown in Figure 4 demonstrate that there was no increase in the sensitivity to CP following FIR irradiation below the lethal temperature, suggesting that the residence time in the high temperature surface region was too short to induce increased bacterial sensitivity to CP, although long enough for damage that affects the sensitivity to RFP.

We have previously measured bacterial monochromatic absorption coefficients in the infrared range [6]. The values of coefficients of *E. coli* used in this work are almost identical to those of water, indicating that the bacteria are optically equivalent to water in the far-infrared range. Therefore, unlike ultrasonic sterilization [1,20], it is difficult to think that the local intracellular temperatures become extremely higher than extracellular temperature. Bacteria circulating in the suspension will, however, be subjected to repeated episodes of rapid temperature fluctuation as they pass to and from the surface region. The accumulated effect of this repeated stress might cause damage and death even when the bulk temperature of the suspension irradiated by FIR is kept below the lethal temperature.

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