Effects of Low-Power He-Ne Laser on the Growth of Yeast under High Pressure

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The effects of He-Ne laser irradiation on the growth of yeast were investigated by counting viable cells at various temperatures (15 - 43°C) and pressures (0.1, 50, 100, and 150 MPa). The laser irradiation was found to be able to retard the growth inhibitory effect of hydrostatic pressure thus stimulating the growth of yeast cells under pressures up to 100 MPa. [He-Ne laser, biostimulating effects, yeast, growth thermogram]

1. Introduction

Laser radiation has been widely and variedly used in medicine and biology [1,2]. High-power laser radiation can destroy normal and neoplastic tissues. On the other hand, low-power He-Ne laser (soft laser) is widely used in the field of medicine for the treatment of postsurgical headaches, post-therapeutic neuralgia (PTN), failure of skin ulcers to heal, rheumatic arthritis, and bronchial asthma. It is known that the irradiation of low-power visible monochromatic red light such as He-Ne laser often stimulates metabolic systems in various cells and accelerates the growth. Irradiation of laser caused stimulation of DNA synthesis in cell and enhanced cell division rate.

On the contrary, high hydrostatic pressure acts as stresses to the organisms living under atmospheric pressure and gives various effects to their physiology and ecology. Generally the growth of common microorganisms (e.g., *E. coli* and yeasts) is retarded by pressurization up to 50 MPa and it often leads microorganisms to death at higher pressures than 50 MPa. We have studied the behaviors of microorganisms under high pressure [3-6]. The retardation of the growth is one of the bottle necks for the studies and the application of microorganisms (e.g., alcoholic fermentation) under high pressure. The purpose of this paper is to check whether low-power He-Ne laser irradiation can moderate or conquer the negative effects of high pressure on the growth of yeast.

2. Materials and methods

2.1. Preparation of cultures

The yeast *Saccharomyces cerevisiae* IFO 10149 was grown at 30°C in YPD medium containing (g/l) glucose, 20; polypeptone, 20; and yeast extract 10. Cultures in the logarithmic phase of growth were used. The logarithmic phase cells were suspended in fresh YPD medium and irradiated for 0 - 120 min under various pressures up to 150 MPa at temperatures of 15 to 43°C. A part of the suspensions was used to count viable cell numbers using YPD agar culture. The experiments were repeated at least three times and the average values were used as survival ratios.

2.2. Measurements of metabolic heats of irradiated yeasts

The metabolic heats of irradiated yeasts were measured using a microcalorimeter (Calorimetry Science Company, DSC

4100) at 30°C. The yeast suspensions were irradiated for desired time with a He-Ne laser (8.5 mW, wavelength 632.8 nm, Senko Medical Instrument Mfg.) and then put in the ampoule of the calorimeter. It took about two days to get the growth-time curve (growth thermogram).

2.3. High pressure apparatus

A high pressure vessel (130×130×130 mm) with optical windows was used for the irradiation of laser under high pressures up to 150 MPa (Fig.1). The light source of He-Ne laser was a Nippon Electric Co. product (GLG5260, 5 mW). Optical power of laser was measured by ADVANTEST optical power multimeter (Q8221, Q82214 type). The temperature of the sample was kept constant by circulating water from thermostat.

3. Results and discussion

3.1. Confirmation of the biostimulating effects of laser by calorimetry

Much work has been made by many investigators to study quantitatively the biological activities of living cells and tissues by observing their metabolic heats [7]. The method is mainly based on the fact that the heat effect is strictly proportional to the

Fig. 1. High pressure system with He-Ne laser.
1. High pressure pump. 2. Bourdon gauge. 3. High pressure vessel. 4. Inner sample cell. 5. Sapphire window. 6. Water bath. 7. Optical power meter. 8. He-Ne laser system.

Fig. 2. Effects of low-power He-Ne laser irradiation under high pressure on the growth of yeast cells at 30 °C. Numbers of viable cells were determined by counting colonies on nutrient medium. N, colonies after incubation; N_0 , colonies before incubation.

Applied pressure: A, 0.1 MPa; B, 50 MPa; C, 100 MPa. Irradiation time: $\triangle$, 0 min (nonirradiated); $\diamond$, 0 - 60 min; $\circ$, 0 - 120 min (full time).

metabolic activity and in most cases a magnitude of calorimetric signal which is given by power unit (μ watt) is simply employed as an index to express the biological activities.

In order to check the dependence of irradiation time, the ratio of the metabolic heats of irradiated and nonirradiated yeasts was plotted against irradiation time. There was a clear stimulation effect of laser for the yeast irradiated for up to 30 min. However, the irradiation for more than 60 min did not accelerate the growth of yeast any more.

3.2. Laser stimulation effects under high pressure

The maximum biomass in yeasts is accumulated at a wavelength of 632.8 nm of He-Ne laser. Figure 2 shows the effects of pressure and irradiation of He-Ne laser on the growth of yeast cells at 30°C. At atmospheric pressure (Fig.2(A)), yeast cells irradiated with He-Ne laser showed similar pattern to that of nonirradiated yeast, although a small effect of the irradiation was found for 120 min irradiation. At 50 MPa (Fig.2(B)), the growth of nonirradiated yeasts was inhibited, however, the viable cell num-

bers of irradiated ones were increased. The rate of increase in the viable cell numbers corresponded to that of the nonirradiated ones at 0.1 MPa. In spite of the laser irradiation, the viable cell numbers decreased at 100 MPa, although they were higher than those of the nonirradiated yeasts (Fig.2(C)). The effect of laser irradiation completely disappeared at pressures higher than 150 MPa.

Figure 3 shows the effects of the laser irradiation on the growth at various temperatures (15 - 43°C) and pressures (0.1, 50, and 100 MPa). All pressurization time is 120 min. At about 30°C which is the optimum temperature concerning the growth of yeast at atmospheric pressure, the viability became maxima under almost all conditions. At 0.1 MPa (Fig.3(A)), viable cell numbers of irradiated yeasts are larger than those of nonirradiated ones, although the results of the irradiation time of 60 and 120 min are almost same. The effects of laser irradiation were largest at 50 MPa (Fig.3(B)); especially at 25 - 30°C the ratio of the viable cell numbers after and before incubation became more than two times that of nonirradiated one. Since 50 MPa is a limit of the growth of yeast, nonirradiated cells do not grow. However, the cells

Fig. 3. Effects of low-power He-Ne laser irradiation on the growth of yeast cells at various temperatures and pressures.

N , colonies after incubation; N_0 , colonies before incubation.

Applied pressure: A, 0.1 MPa; B, 50 MPa; C, 100 MPa.

Irradiation time: $\triangle$, 0 min (nonirradiated); $\diamond$, 0 - 60 min; $\circ$, 0 - 120 min (full time).

Pressure and temperature were applied for 120 min.

irradiated for 120 min were stimulated about 2.3 times of N_0 , the value of (N/N_0) correspond to that of the nonirradiated one at 0.1 MPa. This means that laser irradiation completely compensates the retardation of the growth of yeast due to pressurization of 50 MPa. Even at 100 MPa (Fig.3(C)), the viable cell numbers of irradiated yeast increased at 25 - 30°C.

As mentioned above, it was found that He-Ne laser irradiation could moderate or compensate the effects of high pressure, high temperature, and low temperature which often hinder the growth of yeast cells. Karu reported that when some yeasts were irradiated by He-Ne laser, the protein syntheses in the cells were most accelerated at the dose of 4×10^{-3} J/m² [1]. Although we used 10 - 100 times higher dose than Karu *et al.* used, the biostimulating effects of laser on yeast cells were confirmed under pressures up to 100 MPa.

The mechanisms of biostimulating effects of laser are not yet clear. One of the explanations is that respiratory chain components are important as primary photoacceptors and the absorbed light quanta induces the changes in the redox state of the respiratory chain [8]. Other possibilities are the effects of laser on the chromophores which compose pigments in cells and accept photons, and the effects on the proteins which support the chromophores.

Low-power He-Ne laser does not always stimulate the metabolism of organisms. Long time irradiation (high dose) often damages photoacceptors and causes the inhibition of metabolism

and even the death of the cells. There are many negative reports which doubt the biostimulating effects of low-power laser in clinical levels. The growth rates of human cells are very slow compared to that of bacterial and yeast cells and the control of growth of tissues or organs is very complex processes compared to that of each cell. So we have to discuss the various phenomena induced by the laser radiation in more detail.

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