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Effects of Microwave Irradiation on Growth in *E. coli* Cultivation

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When *E. coli* were cultured under microwave irradiation, an increase in the number of bacteria was observed compared to the conventional method, which was assumed to be caused by the disruption of the quorum sensing system, a signaling mechanism of microorganisms. The growth of *E. coli* was observed in the culture medium containing auto-inducer signaling molecules involved in the quorum sensing system, and a decrease in growth was observed after microwave irradiation.

We have previously shown that microwave heating promotes the growth of bacteria such as *Escherichia coli* and *Bacillus subtilis*. The growth curve of microorganisms goes through a logarithmic phase to a stationary phase, and it was found that the number of bacteria in the stationary phase increased by about 1.5 times under microwave irradiation. The stationary phase is conventionally considered to be an equilibrium between newborn and dead microorganisms, but recent research has revealed that the quorum sensing system functions to control the number of microorganisms. The quorum sensing system is a system in which microorganisms express a certain amount of a signaling molecule called an autoinducer and communicate between cells. If the number of bacteria increases, the number of autoinducers also increases, which stops the growth of the bacteria and keeps the number of bacteria. This is thought to be due to the fact that the number of cells does not increase beyond the necessary level in order to adapt to a lack of nutrient sources or sudden changes in the environment. However, as the number of fungi increased under microwave irradiation compared to normal culture, we hypothesized that the quorum sensing system may have been disrupted.
To test this hypothesis, the following experiments were carried out. *E. coli* cultures under normal conditions were centrifuged, the supernatant was assumed to contain autoinducers, to which a new nutrient source was added and the bacteria were cultured under microwave irradiation as a culture medium. For specific experimental manipulations, 10 µl of *E. coli* were added to 10 ml of LB liquid medium, stirred and incubated at 37 °C. Sampling was done every 2 h and turbidity was measured at a wavelength of 600 nm (day 1 in **Fig. 1**). The culture medium, incubated until stationary state, was centrifuged at 5000 rpm for 5 min to separate the bacteria. 5 ml of the supernatant of the culture medium was taken, transferred to a new sterile test tube, added 5 ml of fresh LB liquid medium and 10 µl of *E. coli*, stirred and incubated under conventional and microwave irradiation (day 2 in **Fig. 1**). Both temperature settings were carried out at 37 °C, the optimal temperature for *E. coli*. The conventional incubation was carried out in a water bath, while the microwave-irradiated incubation was carried out in a precisely temperature-controlled microwave-irradiated apparatus manufactured in our laboratory. Each was sampled every two hours and turbidity was measured at a wavelength of 600 nm and compared. Turbidity decreased in both conventional and microwave-irradiated cultures due to the presence of autoinducers, but only by 7% in the conventional culture and by 3% in the microwave-irradiated culture. This result confirmed growth despite the high amount of autoinducers present. As the molecular structure of the auto-inducers in *E. coli* has been determined, quantitative experiments using these signaling molecules should be carried out in the future.

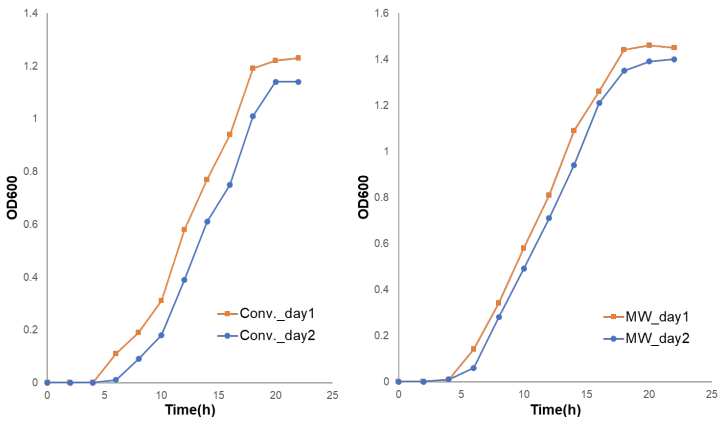

Figure 1. Growth of *E. coli* by conventional and microwave heating.