Simultaneous Cell Analysis By Pt-Mesh Enzyme Electrode And Clsm Image

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Several kinds of microorganisms are used in the fields of food processing and purifying facility. The control of microorganism species in the incubator by external stimulus such as ultrasonic treatment (UT) has been required from the food quality and sanitation points of view. Recently, Confocal Laser Scanning Microscope (CLSM) has been applied to take the individual images of fluorescence stained organelles in the cell [1]. On the other hand, the biosensor with a biocatalyst would be detecting released substances from microorganisms [2].

In this research, the effect of UT on *Saccharomyces cerevisiae* (yeast) was evaluated by the CLSM imaging of simultaneous stained organelles with fluorescence reagent, and by measuring ethanol as metabolism product of yeast by using a Pt-mesh enzyme electrode with alcohol dehydrogenase (ADH). Furthermore, the CLSM imaging and the alcohol biosensor were also applied for evaluating the characteristics of yeast in nitrogen atmosphere with or without UT.

CLSM was applied for the microscopic examination of yeast. The profile of yeast organelles was successfully imaged by differential interference microscopy method (DIC). Endoplasmic reticula stained with MDY-64 (ex.: 456nm) and mitochondria with Rhodamine B (ex.: 556nm) were observed with fluorescent microscopic approach, respectively. The organelle collapse phenomenon without the cell burst by the UT (28 kHz) exposure for 10 minutes was possible to observe as both the increases in the intensity and the extensity of fluorescent stained area in the cell.

The alcohol biosensor consisted of the Pt-mesh electrode and ADH. The ADH was immobilized onto the Pt-mesh electrode surface by chemical crosslinking process with glutaric aldehyde solution. A fixed potential of -650 mV vs. Ag was applied to the Pt-mesh working electrode by a potentiostat to detect NADH as electron acceptor for ADH enzymatic reaction with ethanol. The alcohol biosensor with 2.0 mmol/l NAD^+ was used to measure ethanol from 0.1 to 10.0 mmol/l with a correlation coefficient of 0.995.

The CLSM imaging of mitochondria stained with fluorescence particle G25 (ex.: 458nm) and the mesh-type alcohol sensor were applied for evaluating the characteristics of yeast in nitrogen atmosphere with or without UT. The biosensor was possible to monitor the ethanol increase as the product of an-aerobic metabolism with no CLSM fluorescence change in the yeast without UT. On the contrary, the CLSM fluorescence increased with no alcohol production in the sample damaged by UT.

REFERENCES

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