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Determining Cell Viability of Algal Protoplasts Using the CVC-kit

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Determining cell viability is essential to plant tissue culture in many ways. Usually cell viability of algal protoplasts is determined by microscopic assessment of cytoplasmic features. However, sometimes this method is insufficient to clear-cut evaluation of protoplast viability. Various methods for determining protoplast viability have been known in higher plants.[1] It is desirable to have a fast and short-term method for assessing viability of algal protoplasts, but there is few such a convenient method in marine macro-algae to date. The purpose of the present paper is to examine various staining-dyes in order to develop simple technique for determining protoplast viability using the CVC-kit in marine macro-algae.

The material used in this study was unialgal culture strain of the marine green alga *Ulva pertusa* (gametophyte, female strain) which was obtained in a similar way described as before.[2] The cell cultures were subcultured every 3 months for several years in 200 ml plastic vessel containing 180 ml of ASS_1 medium.[3] Protoplasts were isolated according to the method described previously.[4] The protoplasts were collected and resuspended in hypertonic seawater (supplemented with 0.7 M sorbitol, 10 mM HEPES, pH 8.0) at a density of 2×10^4 protoplasts per ml. Then 0.05 ml of protoplast suspension was transferred into the CVC-kit and immersed in the dye solutions at 20°C for 20 min. The CVC-kit (**Fig. 1**) was prepared by the following procedures: the staining-dyes, 0.01% concentration, were dissolved in the hypertonic seawater; adjusted the pH to 8.0 and put into each well of a plastic vessel (Cell Wells ♯25860, Corning) up to 0.25 ml. The compounds tested were Evans blue, phenosafranin, eosin Y, erythrosine, neutral red and FDA. The CVC-kit was stocked at 5°C in the dark before use. After immersion, they were rinsed several times with the hypertonic seawater and transferred onto a glass slide, then they were observed with a microscope. The FDA-treated protoplasts were observed with the same microscope by using a fluorescence system.

Evans blue, phenosafranin, eosin Y and erythrosine stained inviable protoplasts of the organisms, and phenosafranin stained them especially deeply. But these staining-dyes did not stain viable protoplasts of the organisms. On the other hand, neutral red and FDA stained the viable protoplasts. But these staining-dyes did not stain the inviable protoplasts. According to the results of the present study, Evans blue, phenosafranin, eosin Y and erythrosine were suitable for the detection of inviable protoplasts, and neutral red and FDA were suitable for the detection of viable protoplasts. The most suitable staining-dyes tested in the present study were phenosafranin for the detection of inviable protoplasts (**Fig. 2**) and neutral red or FDA for the detection of viable protoplasts (**Figs. 3, 4**).

The present method by use of CVC-kit allows a rapid and convenient test of cell viability, and it will be promising to develop efficient methods for marine-biotechnology, especially mutagenesis or freeze preservation in marine macro-algae.

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Abbreviations: CVC-kit, cell viability checking kit; FDA, fluorescein diacetate.

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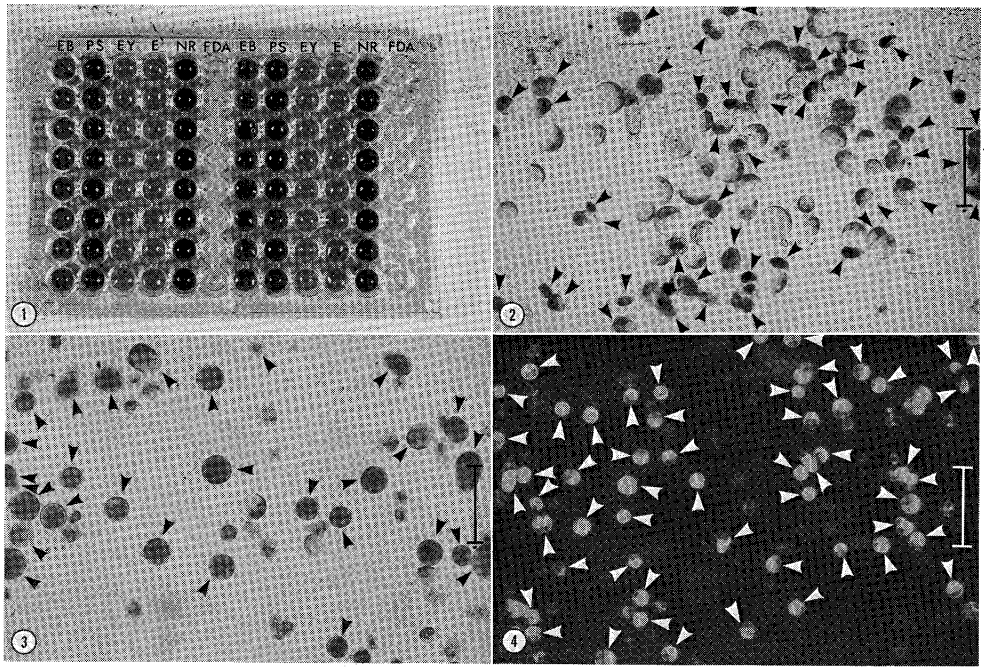

Fig. 1. CVC-kit. EB, Evans blue ; PS, phenosafranin ; EY, eosin Y ; E, erythrosine ; NR, neutral red ; FDA, fluorescein diacetate.

Fig. 2. Staining with phenosafranin in *Ulva pertusa*. Phenosafranin stained only inviable protoplasts purple-red color. Arrowheads indicate inviable protoplasts. Scale bar represents 20 μm.

Fig. 3. Staining with neutral red in *U. pertusa*. Neutral red stained only viable protoplasts crimson color. Arrowheads indicate viable protoplasts. Scale bar represents 20 μm.

Fig. 4. Staining with FDA in *U. pertusa*. The viable protoplasts treated with FDA radiated yellow-green fluorescence, and the inviable protoplasts radiated red fluorescence (due to chlorophyll) or did not radiate any fluorescence. Arrowheads indicate viable protoplasts. Scale bar represents 40 μm.

References

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細胞生存率評価キットによる藻類プロトプラスト生存率の決定

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海産緑藻アナアオサの単藻培養体からプロトプラストを調製し，数種の染色剤の入ったマイクロプレート CVC-kit（細胞生存率評価キット）を適用して有効な染色剤を調べた．その結果，生細胞の検出にはニュートラルレッドやフルオレセインジアセテートが，死細胞の検出にはフェノサフラニンがもっとも有効であり，大型海藻のプロトプラストの生存率を CVC-kit を用いて簡易に評価できることが解った．