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Increasing the Membrane Permeability of a Fern with DMSO

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[Abstract] Cell membrane prevents the entrance of extra molecules (*e.g.*, transcription and translation inhibitors) into the cell. For studying the physiological effects of transcription and translation inhibitors on *Hymenophyllum caudiculatum* fronds, we incubate fronds with 0.1% DMSO to test if this increases cell membrane permeability relative to incubation with ultrapure water. The study showed that DMSO could significantly improve the cell membrane permeability of filmy fronds.

Keywords: Filmy ferns, Membrane permeability, DMSO, Propidium iodide

[Background] One of the most remarkable characteristics of filmy ferns is that the frond, apart from the vascular tissue, is made of a single cell layer and lacks stomata. In our previous work (Garcés *et al.* 2018), we incubated *Hymenophyllum caudiculatum* fronds with cycloheximide or actinomycin D to study the effects of translation, or transcription inhibition respectively. If an exogen inhibitor incubated with a filmy fern frond does not affect plant physiology, it may be because the inhibitor fails to enter the cell. In order to improve the entrance of inhibitors into the cell, we tested if an aqueous solution of 0.1% DMSO has effects on cell membrane permeability, visualizing the entrance of propidium iodide (PI) into the cells.

Materials and Reagents

1. 1.5 ml microcentrifuge tubes
2. Pipette tips
3. Plain slides 75 x 25 mm (VWR, catalog number: 48300-026)
4. Micro cover slides, square 22 x 22 mm (VWR, catalog number: 48366-067)
5. Filter (0.22 µm)
6. DMSO (Sigma-Aldrich, catalog number: D8418)
7. Propidium iodide (PI) 10 mg (Sigma-Aldrich, catalog number: P4170)
8. Ultrapure water 18.2 MΩcm
9. PI (Propidium Iodide) stock (see Recipes)
10. PI-H_2O control (see Recipes)
11. PI-DMSO solution (see Recipes)

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Equipment

1. Pipettes
2. Confocal laser scanning microscope (Olympus, model: Fluoview FV1000)
3. Ultrapure water system (Elga LabWater, model: PureLab Classic)

Software

1. FV10ASW v.2.0c
2. ImageJ 2.0.0-rc-41/1.50d

Procedure

1. Performing the permeability test in 1.5 ml microcentrifuge tubes
   a. Add 1 ml PI-DMSO solution (Recipe 3) and PI-H_2O control (Recipe 2) into different 1.5 ml microcentrifuge tubes. Put freshly obtained, similar size about 1 cm of pinnae into the tubes. Incubate at room temperature.
   b. After 5 min, 30 min, 2 h, and 5 h take out the pinnae, rinse briefly in distilled water to remove residual PI, and then mount on slides to observe fluorescence under Confocal microscope.
2. Confocal microscopy
   a. Observe the Intracellular PI fluorescence under a Fluoview FV1000 confocal laser scanning Biological Microscope (Olympus, Japan). The software FV10ASW v.2.0c and ImageJ 2.0.0-rc-41/1.50d are used to capture the images and construct Z projections. Detect chlorophyll autofluorescence using excitation and emission wavelengths of 488 nm and 687 nm, respectively, PI using excitation and emission wavelengths of 538 and 619 nm, respectively.
   b. We found that after 30 min of incubation, the PI had entered the cells (Figure 1). Images were obtained from three samples.

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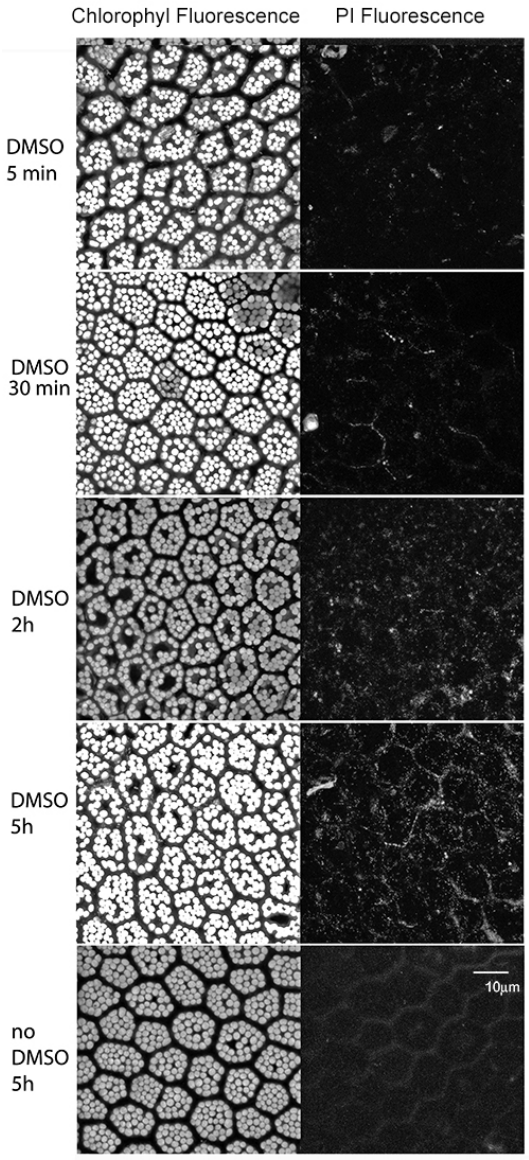

Figure 1. Permeability test for *Hymenophyllum caudiculatum* fronds. *Hymenophyllum caudiculatum* fronds were incubated with 0.1% DMSO or distilled water (control) with 1.5 mM PI. Fluorescence of the chlorophyll (left panel) or PI (right panel) is observed. The same field of view was recorded at different wavelengths.

Data analysis

Three samples were analyzed and representative results are shown in Figure 1. After 30 min of incubation with 0.1% DMSO, PI fluorescence starts being visible inside the cells and reaches the maximum after 5 h. In contrast, PI fluorescence was almost invisible after 5 h incubation of PI with ultrapure water.

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Recipes

1. PI (Propidium Iodide) stock solution (1 mg/ml or 1.5 mM)
   To prepare, add 10ml distilled water to 10 mg PI. Store 1 ml aliquots in 1.5 ml tubes at -20 °C, protecting from light
2. PI-H_2O control (10 ml)
   1.5 µM propidium Iodide
   ultrapure water
   a. In 10 ml of ultrapure water add 10 µl of 1.5 mM PI (stock solution)
   b. Filter sterilize (0.22 µm) and store at room temperature
3. PI-DMSO solution (10 ml)
   0.1% DMSO
   1.5 µM propidium Iodide
   a. In 10 ml of ultrapure water add 10 µl of DMSO and 10 µl of 1.5 mM PI (stock solution)
   b. Filter sterilize (0.22 µm) and store at room temperature

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Competing interests

The author declares no conflicts of interest or competing interests.

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