Elongation of tobacco BY-2 cells derived from miniprotoplasts

Although spontaneous elongation is not unusual in cultured cells, such cells must be made more readily available if they are to be useful in analyzing cell elongation mechanisms. Here, we introduce an efficient and synchronized cell elongation system using tobacco BY-2 miniprotoplasts. Using this process, the miniprotoplasts in culture begin to elongate 6 hours after preparation, and elongate linearly for 24 hours. The average starting cell length of fresh miniprotoplasts is 24 μm, and the final length reaches 37 μm after 24 hours in culture. The elongation rate is approximately 0.72 μm per hour. Almost all the cells are constantly elongating during this period (semi-synchronous cell elongation).

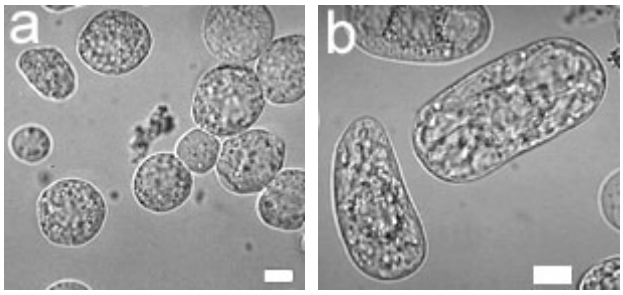

Figure 1. Elongation of tobacco BY-2 miniprotoplast.

DIC images of the cells 0 h (a) and 24 h (b) after miniprotoplast culture. Scale bars: 10 μm.

Procedure

1. To prepare BY-2 protoplasts, 3-day-old cell cultures are incubated at 30°C with an enzyme solution in a water bath for 60 min.
2. Miniprotoplasts are prepared from the incubated BY-2 protoplasts by density gradient centrifugation with Percoll solution at 15,000g for 30 min.
3. Miniprotoplasts are collected from the pellet, washed twice with 0.7 M mannitol and the vacuoles are then collected from the supernatant.
4. Miniprotoplasts, in a state of elongation, are then cultured at 27°C in a modified FMS medium at 5 x 10^4 cells/ml cell density.

Solutions

Enzyme solution

1% cellulase Y-C (Kyowa Chemical Products Co., Ltd., Osaka, Japan)

0.1% pectolyase Y-23 (Kyowa Chemical Products Co.)

0.35 M mannitol

pH 5.5

Percoll solution

30% Percoll (GE Healthcare Ltd, Amersham, UK)

0.7 M mannitol

20 mM MgCl2

Modified FMS medium (for cell elongation, please refer to Hasezawa and Syono, 1983)

1% sucrose

mg/l Murashige and Skoog Plant Salt Mixture (Wako Pure Chemical Industries Ltd., Osaka, Japan)

0.001% thiamine-HCl

0.1% myo-inositol

5.4 x 10^{-7} mM naphthalene acetic acid

4.4 x 10^{-6} mM benzyladenine

0.5 M mannitol

References

Hasezawa S, Syono K (1983) Hormonal control of elongation of tobacco cells derived from protoplasts. *Plant and Cell Physiology* 24:127-132.

Sonobe S (1990) Cytochalasin B enhances cytokinetic cleavage in miniprotoplasts isolated from cultured tobacco cells. *Protoplasma* 155:239-242.

Okubo-Kurihara E, Sano T, Higaki T, Kutsuna N, Hasezawa S (2009) Acceleration of vacuolar regeneration and cell growth by overexpression of an aquaporin NtTIP1;1 in tobacco BY-2 cells. *Plant and Cell Physiology* 50:151-160.