Maintenance of *Arabidopsis thaliana* T87 cell suspension culture

Introduction

T87 cell suspension culture was obtained from a seedling of *Arabidopsis thaliana* L. (Heynh.) ecotype Columbia (Axelos *et al*. 1992). The culture is composed of small, near-uniform clumps of cells, has a green color (Figure1), and is maintained in Jouanneau and Péaud-Lenoël (JPL) medium under continuous light at 22˚C and subcultured at two-week intervals.

Materials

I. Stock Solutions and Chemicals

A) JPL A' (1 L)

KNO_3	65.5 g
$CaCl_2 \cdot 2H_2O$	4.4 g
$MgSO_4 \cdot 7H_2O$	3.7 g
KH_2PO_4	1.7 g

B) JPL B (1 L)

H_3BO_3	6.2 g
$MnSO_4 \cdot 4H_2O$	22.3 g
$ZnSO_4 \cdot 7H_2O$	10.6 g
KI	0.83 g
$Na_2MoO_4 \cdot 2H_2O$	0.25 g
$CoCl_2 \cdot 6H_2O$	0.025 g
$CuSO_4 \cdot 5H_2O$	0.025 g

C) JPL C (1 L)

$FeSO_4 \cdot 7H_2O$	2,780 mg
Na_2-EDTA	3,730 mg

D) JPL D (1 L)

myo-Inositol	10 g
Glycine	0.2 g

E) JPL VT (100 mL)

Nicotinic acid	50 mg
Pyridoxine·HCl	50 mg
Thiamine·HCl	40 mg

F) JPL P (100 mL)

200 mM KH_2PO_4	19.5 ml
200 mM Na_2HPO_4	30.5 ml
H_2O	50 ml

G) 1 mM NAA (100 mL)

1-Naphthaleneacetic acid	18.62 mg

H) Casein hydrolysate, vitamin-free
Casamino acids vitamin assay, Difco (228820)

II. Glassware and Stainless Sieves
（All are sterilized by autoclaving at 121˚C for 20 min.）

A) Erlenmeyer flask (300 ml), capped with two layers of aluminum foil
B) Pipette, large tip opening (10 ml), and a bulb
C) Stainless sieve (diameter, 5 cm; pore size, 1 mm) set on a tall beaker (200 ml), capped with two layers of aluminum foil

III. Preparation of JPL Medium

A) Prepare three solutions as follows:

A)-1 JPL mineral solution (1 L)

JPL A'	37.5 ml
JPL B	0.375 ml
JPL C	2.5 ml

Adjust pH to 5.7 with 0.2 N KOH.

A)-2 JPL organic solution (100 mL)

Casein hydrolysate	0.1 g
JPL D	10 ml
JPL VT	1 ml

Adjust pH to 5.7 with 0.2 N HCl.

A)-3 JPL sucrose solution (100 mL)

Sucrose	15 g
JPL P	1 ml
1 mM NAA	1 ml

B) Autoclave these solutions at 121˚C for 20 min.
C) Add 800 ml of JPL mineral solution, 100 ml of JPL organic solution, and 100 ml of JPL sucrose solution aseptically.
D) Pour 80 ml of JPL medium into a sterile flask.

Methods

I. Filter a two-week-old cell suspension through a stainless sieve (Figure 2).
II. Agitate the filtrate well and transfer 2 ml of cell suspension to 80 ml of fresh JPL medium with a pipette.
III. Incubate cell cultures on a rotary shaker at 120 rpm under continuous light (40–100 μE/s/m^2) at 22˚C.

Notes

I. T87 cells may be maintained in other media. We have culture them in JPL medium as described in the original report by Axelos *et al.* (1992). Yamada *et al.* (2004) reported that T87 cells grew well in Gamborg's B5 medium. When T87 cells are cultured in the medium other than JPL medium, it is necessary to optimize the subculture method, *e.g.*, subculture interval, amount of cells transferred, and the pore size of the sieve.

II. T87 cells should be transferred to fresh medium immediately after arrival. We transport T87 cells on semi-solid JPL medium in 250-ml disposable flasks. T87 cells should be collected from the medium with a spatula and transferred to Erlenmeyer flasks containing fresh liquid medium. The cell suspension culture should be carefully established from small scale fractions of the original culture (*e.g.*, 20 ml of medium in a 100-ml flask) because some T87 cells may have been damaged during transport.

III. The density of living cells subcultured to fresh medium is important for stable maintenance of cell suspension cultures. A high initial density causes overgrowth after the usual culture period, while a low density suppresses cell division. Occasionally, T87-cell clumps develop into large aggregates, which cause a decrease in the number of cells passed through a 1-mm sieve. In such cases, it should be confirmed that an adequate amount of cells are transferred to each fresh medium during subculturing.

IV. Under our culture conditions, the mass of T87 cells had increased around 50-fold by day 14 of culturing (Figure 3). In addition, T87 cells proliferated in the dark.

References

Axelos M, Curic C, Mazzolini L, Bardet C, Lescure B (1992) A protocol for transient gene expression in *Arabidopsis thaliana* protoplasts isolated from cell suspension cultures. Plant Physiology and Biochemistry 30: 123-128

Yamada H, Koizumi N, Nakamichi N, Kiba T, Yamashino T, Mizuno T (2004) Rapid response of *Arabidopsis* T87 cultured cells to cytokinin through His-to-Asp phosphorelay signal transduction. Bioscience, Biotechnology, and Biochemistry 68: 1966-1976

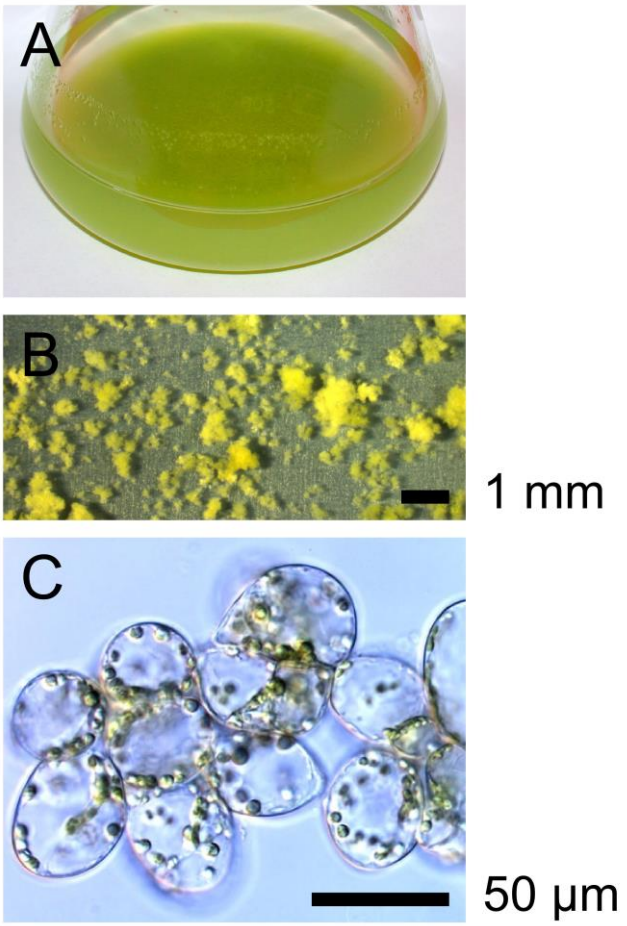

Figure 1 *Arabidopsis thaliana* T87 cell suspension culture

(A) Two-week-old cell suspension culture.
(B) Cell clumps. The size of cell clumps are varied, but most of them are below 1 mm.
(C) Microscopic observation of T87 cells. The cells contain many chloroplasts.

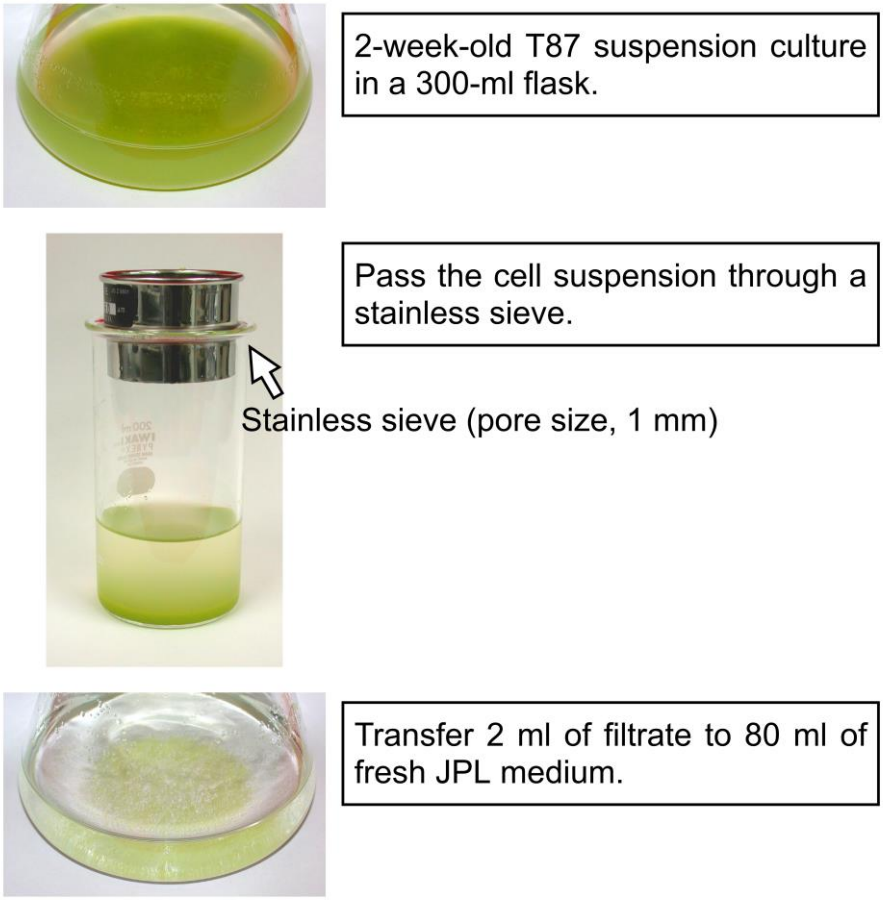

Figure 2 Procedure for subculturing T87 cells

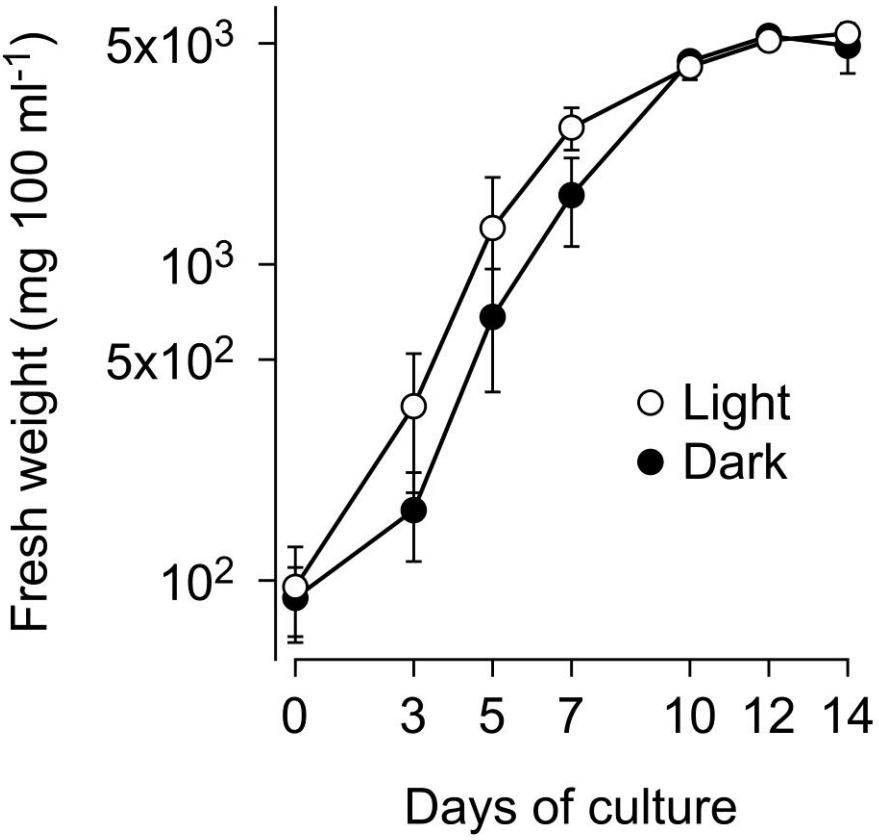

Figure 3 Growth profiles of T87 cells