rpc00008

Arabidopsis thaliana T87 cell suspension culture

Components

- Domestic delivery: A 50-mL plastic conical centrifuge tube, containing cell suspension
- Overseas delivery: A 250-mL plastic Erlenmeyer flask, containing cells placed on semi-solid medium

Notice

- Subculture the cells to fresh medium immediately after arrival [Notes I, II].
- Do not store the cell culture in a refrigerator and a freezer.
- Maintain aseptic conditions of the cell culture, and work in a laminar flow cabinet.

Method

- Culture medium: JPL medium, 1 μM NAA (medium no. 5) [Materials III]
- Culture conditions: 22℃, continuous light, 120 rpm [Methods II]
- Subculture: 14-day intervals [Methods I]

Citation of cell line

When results obtained by using this cell line are published in a scientific journal, it should be cited in the following manner: "*Arabidopsis thaliana* T87 cell line (rpc00008) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan."

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Introduction

Arabidopsis T87 cell line was established from a seedling of *Arabidopsis thaliana* (L.) Heynh. accession Columbia (Axelos *et al.* 1992). The T87 cell culture is green and composed of small near-uniform aggregates of cells (Figure 1). The T87 cells are grown in a Jouanneau and Péaud-Lenoël (JPL) medium supplemented with 1 µM 1-naphthaleneacetic acid (NAA). Our T87 cell culture has been maintained under the continuous light at 22℃ with rotary shaking at 120 rpm and subcultured at 14-day intervals (Figure 2).

Materials

Chemicals and stock solutions

(All stock solutions are stored at 4℃)

A) JPL_A'

KNO_3	65.5 mg/mL
$CaCl_2 \cdot 2H_2O$	4.4 mg/mL
$MgSO_4 \cdot 7H_2O$	3.7 mg/mL
KH_2PO_4	1.7 mg/mL

B) JPL_B

H_3BO_3	6.2 mg/mL
$MnSO_4 \cdot 5H_2O$	24.1 mg/mL
$ZnSO_4 \cdot 7H_2O$	10.6 mg/mL
KI	0.83 mg/mL
$Na_2MoO_4 \cdot 2H_2O$	0.25 mg/mL
$CuSO_4 \cdot 5H_2O$	0.025 mg/mL
$CoCl_2 \cdot 6H_2O$	0.025 mg/mL

C) JPL_C

$FeSO_4 \cdot 7H_2O$	2.78 mg/mL
Na_2-EDTA	3.73 mg/mL
Heat at 80℃ for 3–4 hours for chelating Fe	

D) JPL_D

Glycine	0.2 mg/mL
myo-Inositol	10 mg/mL

E) JPL_VT

Nicotinic acid	0.5 mg/mL
Pyridoxine·HCl	0.5 mg/mL

Thiamine·HCl	0.4 mg/mL

F) JPL_P

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

G) NAA (1 mM)

NAA·K	0.224 mg/mL

Potassium 1-naphthylacetate, FUJIFILM Wako Pure Chemical Corporation (#161-04021)

H) Sucrose

I) Casein hydrolysate, vitamin free

Casamino acids vitamin assay, Difco (#228820)

J) KOH (1 N)

K) HCl (1 N)

Glassware and equipment

(All are sterilized by autoclaving at 121℃ for 20 min)

A) Erlenmeyer flask (300 mL), capped with two layers of aluminum foil

B) Pipette (10 mL; large tip opening) 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

Preparation of JPL medium (medium no. 5)

1. Prepare three solutions as follows.

A) JPL mineral solution (1 L)

JPL_A'	37.5 mL
JPL_B	0.375 mL
JPL_C	2.5 mL

Adjust the pH to 5.7 with KOH (1 N)

B) JPL organic solution (100 mL)

JPL_D	10 mL
JPL_VT	1 mL

Casein hydrolysate	0.1 g
Adjust the pH to 5.7 with HCl (1 N)	

C) JPL sucrose solution (100 mL)

JPL_P	1 mL
NAA (1 mM)	1 mL
Sucrose	15 g

2. Autoclave the flask at 121℃ for 20 min.

3. Add 800 mL of JPL mineral solution, 100 mL of JPL organic solution, and 100 mL of JPL sucrose solution asceptically.

4. Pour 80 mL of the medium into a sterile 300-mL flask.

Methods

1. Filter a 14-day-old cell suspension through a stainless sieve (Figure 3).

2. Agitate the filtrate well and transfer 2–3 mL of cell suspension to 80 mL of fresh JPL medium with a pipette.

3. Incubate cell cultures on a rotary shaker at 120 rpm under the continuous light condition (photosynthetic photon flux density 40–42 μmol m^{-2} s^{-1}) at 22℃.

Notes

- For domestic customers: We send T87 cell suspension in a 50-mL disposable conical centrifuge tube. The cells should be transferred to fresh JPL medium immediately after arrival.

- For overseas customers: We send T87 cells placed on semi-solid JPL medium in a 250-mL disposable Erlenmeyer flask. The cells should be transferred to fresh JPL medium immediately after arrival. Collect the cells from the semi-solid medium with a spatula and transfer them to Erlenmeyer flasks containing fresh liquid medium.

- In order to maintain T87 cell suspension cultures stably, it is essential to transfer an adequate amount of cells to fresh JPL medium in every subculture. The amount of cells may vary from one lab to another, because proliferation of T87 cells is affected by culture conditions, such as a room temperature, rotation speed of a rotary shaker, and aeration condition of the culture. T87-cell clumps occationally develop into large aggregates, which causes a descrease in the amount of cells passed through a 1-mm sieve.

- In order to obtain good aeration of a suspension culture, a silicone sponge plug may be used instead of the aluminum foil cap (*e.g.*, cap-type Silicosen; Shin-Etsu Polymer, Tokyo, Japan; https://www.shinpoly.co.jp/en/product/product/medical/plugs.html).

- T87 cells may be maintained in Gamborg's B5 medium (Yamada *et al.* 2004) or Murashige and Skoog medium (Cunillera *et al.* 2000) other than JPL medium.

References

Axelos M, Curie 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.

Cunillera N, Arró M, Forés O, David Manzano, Ferrer A (2000) Characterization of dehydrodolichyl diphosphate synthase of *Arabidopsis thaliana*, a key enzyme in dolichol biosynthesis. FEBS Letters 477: 170–174. DOI: 10.1016/S0014-5793(00)01798-1

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. DOI: 10.1271/bbb.68.1966

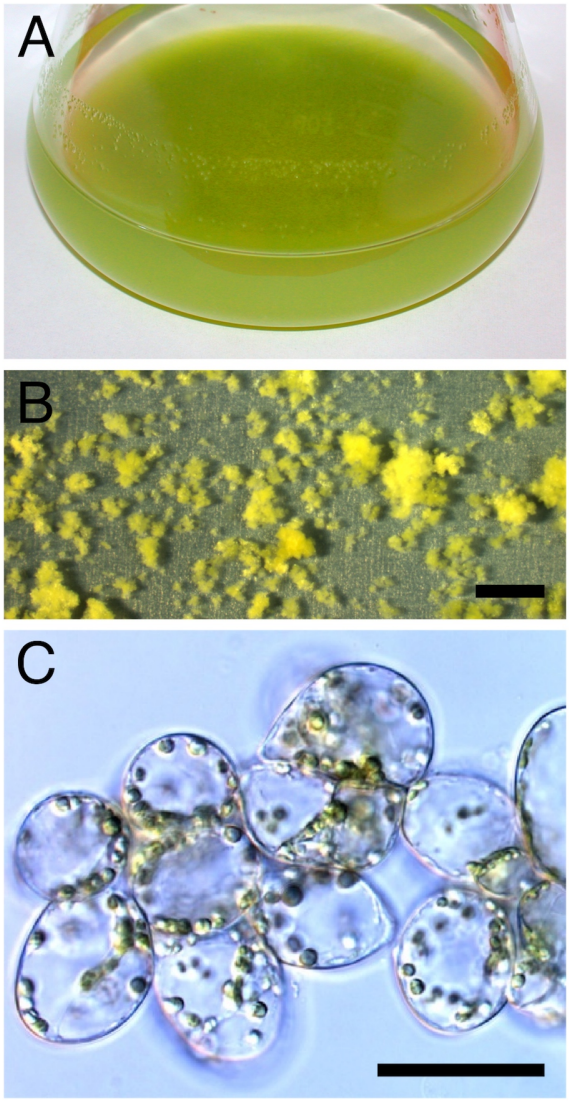

Figure 1. *Arabidopsis thaliana* T87 cell suspension culture

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. Scale bar = 1 mm

C: Morphology of T87 cells. T87 cells were observed by using differential interference contrast microscopy. The cells contain many chloroplasts. Scale bar = 50 μm

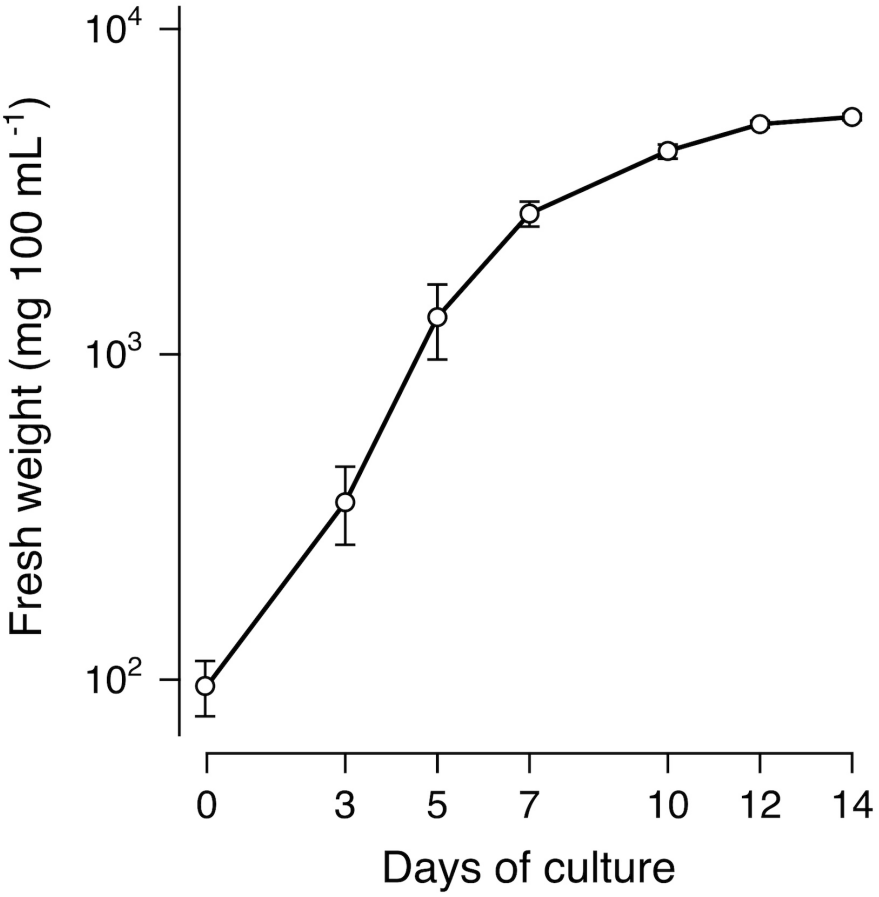

Figure 2. Growth profile of T87 cells

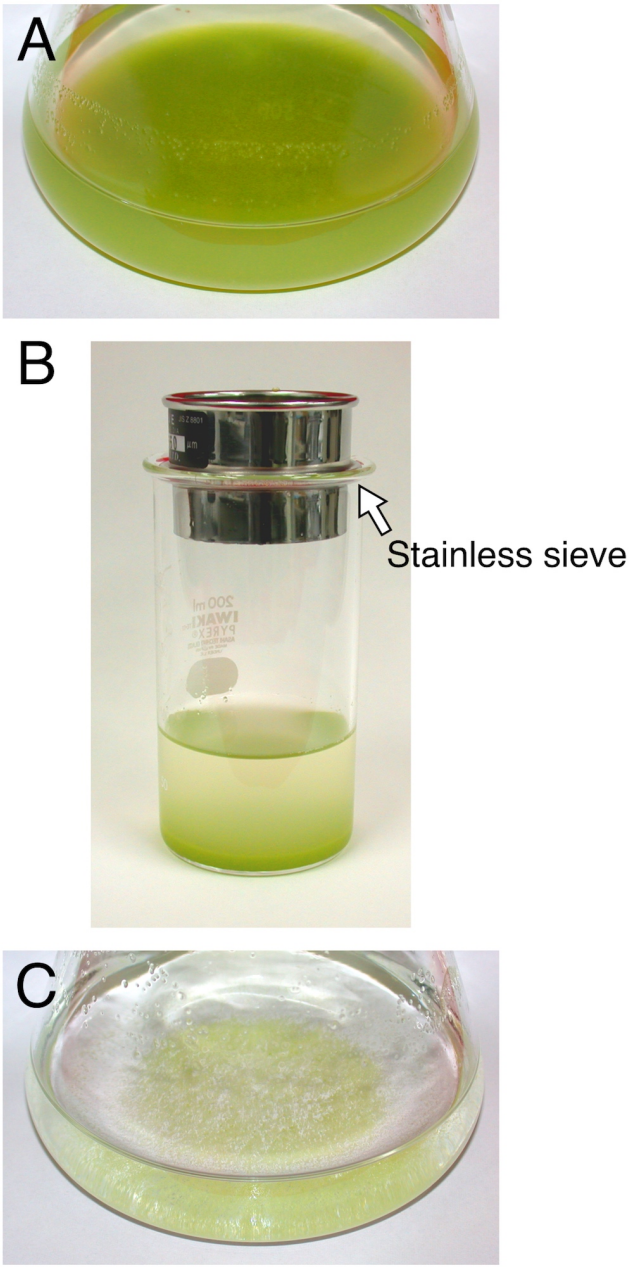

Figure 3. Procedure for subculturing T87 cells

A: Two-week-old cell suspension culture.

B: Pass the culture through a stainless sieve (pore size, 1 mm).

C: Transfer 2 mL of the filtrate to 80 mL of fresh JPL medium.

Appendix A: Formulation of culture medium

Table A.1. Jouanneau and Péaud-Lenoël medium (medium no. 5)

Chemical	Concentration (mg/L)
KNO_3	1965
$CaCl_2 \cdot 2H_2O$	132
$MgSO_4 \cdot 7H_2O$	111
KH_2PO_4	56.308
Na_2HPO_4	8.66
H_3BO_3	1.86
$MnSO_4 \cdot 5H_2O$	7.23
$ZnSO_4 \cdot 7H_2O$	3.18
KI	0.249
$Na_2MoO_4 \cdot 2H_2O$	0.075
$CuSO_4 \cdot 5H_2O$	0.0075
$CoCl_2 \cdot 6H_2O$	0.0075
$FeSO_4 \cdot 7H_2O$	5.56
Na_2-EDTA	7.46
Nicotinic acid	0.5
Pyridoxine·HCl	0.5
Thiamine·HCl	0.4
Glycine	2
myo-Inositol	100
Casein hydrolysate, vitamin free	100
Sucrose	15000
NAA·K	0.224