rpc00110

Vitis vinifera YU-1-c callus culture

Components

- A 9-cm plastic Petri dish, containing cells placed on semi-solid medium

Notice

- Subculture the cells to fresh medium immediately after arrival [Notes I].
- 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: BSS medium, 0.54 µM NAA, 0.2 mg/L kinetin, 0.3% (w/v) gellan gum, pH 5.7 (medium no. 70) [Materials III]
- Culture conditions: 27℃, dark [Methods II]
- Subculture: 21–28-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: "*Vitis vinifera* YU-1-c cell line (rpc00110) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan."

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Introduction

Grape YU-1-c cell line was established by subculturing the YU-1 suspension culture on a semi-solid culture medium. The parent YU-1 cell line (rpc00049) was derived from a shoot apex of *Vitis vinifera* L. cultivar Koshu (Fujita *et al*. 2018). The YU-1-c cells are grown on a BSS medium supplemented with 0.54 µM 1-naphthaleneacetic acid (NAA) and 0.2 mg/L kinetin, and solidified with 0.3% (w/v) gellan gum, pH 5.7. Our YU-1-c cell culture has been maintained in the dark at 27℃ and subcultured at 21–28-day intervals.

Materials

Chemicals and stock solutions

(All stock solutions are stored at 4℃)

A) MS salt mix

Murashige and Skoog Plant Salt Mixture, FUJIFILM Wako Pure Chemical Corporation (#392-00591)

B) Sucrose

C) BSS_VT

Nicotinic acid	5 mg/mL
Pyridoxine·HCl	5 mg/mL
Thiamine·HCl	0.1 mg/mL
Glycine	2 mg/mL

D) MS_inositol

myo-Inositol	40 mg/mL

E) NAA (1 mM)

NAA·K	0.224 mg/mL

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

F) Kinetin (0.2 mg/mL)

Kinetin	0.2 mg/mL

Dissolve kinetin in small volume of KOH (1 N), and fill up with distilled water

G) Gellan gum

Gellan gum, FUJIFILM Wako Pure Chemical Corporation (#073-03071)

H) KOH (1 N)

Glassware and equipment

A) Petri dish (9 cm diameter, 2 cm height), sterile

B) Forceps, sterilized before use

C) Surgical tape

3M™ Micropore™ Surgical Tape, 12.5 mm × 9.1 m, 3M Japan Limited (#1530-0)

Preparation of BSS medium (medium no. 70)

1. Dissolve the following chemicals in approximately 800 mL of distilled water.

MS salt mix	1 bag (1 L)
Sucrose	20 g

2. Add following stock solutions, and fill up to approximately 950 mL with distilled water.

BSS_VT	1 mL
MS_inositol	2.5 mL
NAA (1 mM)	0.54 mL
Kinetin (0.2 mg/mL)	1 mL

3. Adjust the pH of the solution to 5.7 with KOH (1 N), and fill up to 1 L with distilled water.

4. Add 3 g of gellan gum to the medium.

5. Autoclave the medium at 121℃ for 20 min.

6. Pour 30 mL of the medium into a 9-cm Petri dish.

Methods

1. Pick up an appropriate amount of callus cells from a 21–28-day-old culture with a forceps and place the cells onto fresh BSS medium.

2. Seal the Petri dishes using two rounds of surgical tape.

3. Incubate cell cultures under the dark condition at 27℃.

Notes

- We send YU-1-c cells on semi-solid BSS medium in a 9-cm disposable Petri dish. The cells should be subcultured to fresh BSS medium immediately after arrival.

- In order to maintain YU-1-c callus culture stably, it is essential to observe the growth of cells carefully. Because proliferation of YU-1-c cells is affected by culture conditions, such as a room temperature, aeration conditions of the culture and so on, an amount of cells transferred to fresh medium and the subculture intervals may vary from one lab to another. We usually inoculate seven pieces of YU-1-c callus (about 5-mm in diameter) on 30 mL of BSS medium in a 9-cm Petri dish, and culture them for 21–28 days.

- Suspension cultures can be derived from YU-1-c callus cultures by subculturing them into a liquid culture medium. The suspension culture method is the same as rpc00049 YU-1.

References

Fujita K, Aoki Y, Suzuki S (2018) Antidiabetic effects of novel cell culture established from grapevine, *Vitis vinifera* cv. Koshu. Cytotechnology 70: 993–999. DOI: 10.1007/s10616-018-0203-y

Appendix A: Formulation of culture medium

Table A.1. BSS medium
(medium no. 70)

Chemical	Concentration (mg/L)
KNO_3	1900
NH_4NO_3	1650
$CaCl_2 \cdot 2H_2O$	440
$MgSO_4 \cdot 7H_2O$	370
KH_2PO_4	170
H_3BO_3	6.2
$MnSO_4 \cdot 4H_2O$	22.3
$ZnSO_4 \cdot 7H_2O$	8.6
KI	0.83
$Na_2MoO_4 \cdot 2H_2O$	0.25
$CuSO_4 \cdot 5H_2O$	0.025
$CoCl_2 \cdot 6H_2O$	0.025
$FeSO_4 \cdot 7H_2O$	27.8
Na_2-EDTA	37.3
Nicotinic acid	5
Pyridoxine·HCl	5
Thiamine·HCl	0.1
Glycine	2
myo-Inositol	100
Sucrose	20000
NAA·K	0.121
Kinetin	0.2
Gellan gum	3000