rpc00067

Fragaria × *ananassa* SB489 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: mB5 medium, 0.25% (w/v) gellan gum, pH 5.7 (medium no. 53) [Materials III]
- Culture conditions: 25℃, continuous light [Methods II]
- Subculture: 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: "*Fragaria* × *ananassa* SB489 cell line (rpc00067) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan."

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Introduction

Strawberry SB489 cell line was established from a leaf of *Fragaria* × *ananassa* Duchesne cultivar Nyoho (Asano *et al*. 2002). The SB489 callus cells are dark red and produce anthocyanin. The SB489 cells are grown on a phytohormone-free modified Gamborg's B5 (mB5) medium solidified with 0.25% (w/v) gellan gum, pH 5.7. Our SB489 cell culture has been maintained under the continuous light at 25℃ and subcultured at 28-day intervals.

Materials

Chemicals and stock solutions

(All stock solutions are stored at 4℃)

A) B5 salt mix

Gamborg's B5 Medium Salt Mixture, FUJIFILM Wako Pure Chemical Corporation (#399-00621)

B) Sucrose

C) MS_VT

Nicotinic acid	0.5 mg/mL
Pyridoxine·HCl	0.5 mg/mL
Thiamine·HCl	0.1 mg/mL
Glycine	2 mg/mL

D) MS_inositol

myo-Inositol	40 mg/mL

E) Gellan gum

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

F) KOH (1 N)

Glassware and equipment

A) Erlenmeyer flask (200 mL), capped with two layers of aluminum foil

B) Forceps, sterilized before use

Preparation of mB5 medium (medium no. 53)

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

B5 salt mix	1 bag (1 L)
Sucrose	20 g

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

MS_VT	1 mL
MS_inositol	2.5 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. Pour 60 mL of the medium into a 200-mL flask containing 0.15 g of gellan gum.

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

Methods

1. Pick up an appropriate amount of callus cells from a 28-day-old culture with a forceps and place the cells onto fresh mB5 medium.

2. Incubate cell cultures under the continuous light condition (photosynthetic photon flux density 35–40 μmol m^{-2} s^{-1}) at 25℃.

Notes

- We send SB489 cells on semi-solid mB5 medium in a 9-cm disposable Petri dish. The cells should be subcultured to fresh mB5 medium immediately after arrival.

- In order to maintain SB489 callus culture stably, it is essential to observe the growth of cells carefully. Because proliferation of SB489 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 three to five pieces of SB489 callus (about 3–10-mm in diameter) on 60 mL of mB5 medium in a 200-mL flask, and culture them for 28 days.

- Subculture the red SB489 cells on the surface along with the white cells on the inside.

References

Asano S, Ohtsubo S, Nakajima M, Kusunoki M, Kaneko K, Katayama H, Nawa Y (2002) Production of anthocyanins by habituated cultured cells of Nyoho strawberry (*Fragaria ananassa* Duch.). Food Science and Technology Research 8: 64–69. DOI: 10.3136/fstr.8.64

Appendix A: Formulation of culture medium

Table A.1. modified Gamborg's B5 medium
(medium no. 53)

Chemical	Concentration (mg/L)
KNO_3	2500
$(NH_4)2SO_4$	134
$CaCl_2 \cdot 2H_2O$	150
$MgSO_4 \cdot 7H_2O$	250
$NaH_2PO_4 \cdot H_2O$	150
H_3BO_3	3
$MnSO_4 \cdot H_2O$	10
$ZnSO_4 \cdot 7H_2O$	2
KI	0.75
$Na_2MoO_4 \cdot 2H_2O$	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	0.5
Pyridoxine·HCl	0.5
Thiamine·HCl	0.1
Glycine	2
myo-Inositol	100
Sucrose	20000
Gellan gum	2500