rpc00066

Prunus persica P468 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: MS medium, 0.25% (w/v) gellan gum, pH 5.7 (medium no. 44) [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: "*Prunus persica* P468 cell line (rpc00066) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan."

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Introduction

Peach P468 cell line was established from a immature fruit of *Prunus persica* (L.) Batsch cultivar Yamanehakutou (Asano 2011, Asano and Otobe 2011). The P468 callus cells are dark red and produce anthocyanin. The P468 cells are grown on a phytohormone-free Murashige and Skoog (MS) medium solidified with 0.25% (w/v) gellan gum, pH 5.7. Our P468 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) MS salt mix

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

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

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 MS medium (medium no. 44)

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

MS salt mix	1 bag (1 L)

Sucrose	30 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 MS medium.

2. Incubate cell cultures under the continuous light condition (photosynthetic photon flux density 45–50 μmol m^{-2} s^{-1}) at 25℃.

Notes

- We send P468 cells on semi-solid MS medium in a 9-cm disposable Petri dish. The cells should be subcultured to fresh MS medium immediately after arrival.

- In order to maintain P468 callus culture stably, it is essential to observe the growth of cells carefully. Because proliferation of P468 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 P468 callus (about 7-mm in diameter) on 60 mL of MS medium in a 200-mL flask, and culture them for 28 days.

- Dark red P468 cells are suitable for subculture.

References

Asano S (2011) Research into pigment production using cultured plant cells grown without phytohormones. PhD thesis, University of Tsukuba, Japan (in Japanese). http://hdl.handle.net/2241/114680

Asano S, Otobe K (2011) Production of phytochemicals by using habituated and long-term

cultured cells. Plant Biotechnology 28: 51–62. DOI: 10.5511/plantbiotechnology.10.1109a

Appendix A: Formulation of culture medium

Table A.1. Murashige and Skoog medium
(medium no. 44)

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	0.5
Pyridoxine·HCl	0.5
Thiamine·HCl	0.1
Glycine	2
myo-Inositol	100
Sucrose	30000
Gellan gum	2500