rpc00058

Glehnia littoralis GlV 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: B5 medium, 1 mg/L NAA, 0.01 mg/L kinetin, 0.8% (w/v) agar, pH 5.8 (medium no. 48) [Materials III]
- Culture conditions: 27°C, 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: "*Glehnia littoralis* GlV cell line (rpc00058) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan."

Experimental Plant Division
RIKEN BioResource Research Center (BRC)
Koyadai 3-1-1, Tsukuba, Ibaraki 305-0074
Japan
FAX: +81 29 836 9053
E-mail: plant.brc@riken.jp
http://epd.brc.riken.jp/en/

Introduction

GlV cell line was established from a petiole of *Glehnia littoralis* F.Schmidt ex Miq. (Miura *et al*. 1998). The GlV callus culture is violet, and produces anthocyanins (Miura *et al*. 1998) and coumarin derivatives (umbelliferone) (Ishikawa *et al*. 2005). The GlV cells are grown on a Gamborg's B5 medium supplemented with 1 mg/L 1-naphthaleneacetic acid (NAA) and 0.01 mg/L kinetin, and solidified with 0.8% (w/v) agar, pH 5.8. Our GlV 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) B5 salt mix

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

B) Sucrose

C) B5_VT

Nicotinic acid	0.4 mg/mL
Pyridoxine·HCl	0.4 mg/mL
Thiamine·HCl	4 mg/mL
myo-Inositol	40 mg/mL

D) NAA (1 mg/mL)

NAA·K	1.2 mg/mL

E) 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

F) Agar, powder

Agar, powder, Junsei Chemical (#24440-1201)

G) KOH (1 N)

Glassware and equipment

A) Erlenmeyer flask (100 mL), capped with two layers of aluminum foil

B) Forceps, sterilized before use

Preparation of B5 medium (medium no. 48)

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.

B5_VT	2.5 mL
NAA (1 mg/mL)	1 mL
Kinetin (0.2 mg/mL)	0.05 mL

3. Adjust the pH of the solution to 5.8 with KOH (1 N), and fill up to 1 L with distilled water.

4. Pour 40 mL of the medium into a 100-mL flask containing 0.32 g of agar.

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

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 B5 medium.

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

Notes

- We send GlV cells on semi-solid B5 medium in a 9-cm disposable Petri dish. The cells should be subcultured to fresh B5 medium immediately after arrival.

- In order to maintain GlV callus culture stably, it is essential to observe the growth of cells carefully. Because proliferation of GlV 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 four pieces of GlV callus (about 3–5-mm in diameter) on 40 mL of B5 medium in a 100-mL flask, and culture them for 21–28 days.

References

Ishikawa A, Kitamura Y, Ozeki Y, Itoh Y, Yamada A, Watanabe M (2005) Post-stress metabolism involves umbelliferone production in anthocyanin-producing and non-

producing cells of *Glehnia littoralis* suspension cultures. Journal of Plant Physiology 162: 703–710. DOI: 10.1016/j.jplph.2004.11.014

Miura H, Kitamura Y, Ikenaga T, Mizobe K, Shimizu T, Nakamura M, Kato Y, Yamada T, Maitani T, Goda Y (1998) Anthocyanin production of *Glehnia littoralis* callus cultures. Phytochemistry 48: 279–283. DOI: 10.1016/S0031-9422(97)01115-1

Appendix A: Formulation of culture medium

Table A.1. Gamborg's B5 medium
(medium no. 48)

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	1
Pyridoxine·HCl	1
Thiamine·HCl	10
myo-Inositol	100
Sucrose	20000
NAA·K	1.2
Kinetin	0.01
Agar	8000