rpc00035

Nicotiana tabacum Xan-1 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 µM 2,4-D, 0.04 µM kinetin, 0.9% (w/v) agar, pH 5.7 (medium no. 30) [Materials III]
- Culture conditions: 27℃, dark [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: "*Nicotiana tabacum* Xan-1 cell line (rpc00035) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan."

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Introduction

Tobacco Xan-1 cell line was established from a leaf of *Nicotiana tabacum* L. cultivar Xanthi NC (Taguchi *et al*. 2003). The Xan-1 cells are grown on a Gamborg's B5 medium supplemented with 1 μM 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.04 μM kinetin, and solidified with 0.9% (w/v) agar, pH 5.7. Our Xan-1 cell culture has been maintained in the dark at 27℃ 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) 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) 2,4-D (1 mM)

2,4-D sodium monohydrate 0.261 mg/mL
(2,4-Dichlorophenoxy)acetic acid sodium salt monohydrate, Sigma-Aldrich (D6679)

E) Kinetin (1 mM)

Kinetin 0.215 mg/mL
Dissolve kinetin in small volume of KOH (1 N), and fill up with distilled water

F) Agar, powder

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. 30)

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
2,4-D (1 mM)	1 mL
Kinetin (1 mM)	0.04 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 40 mL of the medium into a 100-mL flask containing 0.36 g of agar.

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 B5 medium.

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

Notes

- We send Xan-1 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 Xan-1 callus culture stably, it is essential to observe the growth of cells carefully. Because proliferation of Xan-1 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 pieces of Xan-1 callus (about 3-mm in diameter) on 40 mL of B5 medium in a 100-mL flask, and culture them for 28 days.

References

Taguchi F, Shimizu R, Inagaki Y, Toyoda K, Shiraishi T, Ichinose Y (2003) Post-translational modification of flagellin determines the specificity of HR induction. Plant & Cell Physiology 44: 342–349. DOI: 10.1093/pcp/pcg042

Appendix A: Formulation of culture medium

Table A.1. Gamborg's B5 medium
(medium no. 30)

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
2,4-D sodium monohydrate	0.261
Kinetin	0.0086
Agar	8000