rpc00034

Lotus japonicus LjmA 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, 1 mg/L 2,4-D, 0.1 mg/L kinetin, 0.9% (w/v) agar, pH 5.7 (medium no. 29) [Materials III]
- Culture conditions: 27°C, 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: "*Lotus japonicus* LjmA cell line (rpc00034) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan."

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Introduction

LjmA cell line was established from a mature embryo of *Lotus japonicus* (Regel) K.Larsen accession Miyakojima. The LjmA cells are grown on a Murashige and Skoog (MS) medium supplemented with 1 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.1 mg/L kinetin, and solidified with 0.9% (w/v) agar, pH 5.7. Our LjmA 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) 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) 2,4-D (0.2 mg/mL)

2,4-D sodium monohydrate	0.236 mg/mL

(2,4-Dichlorophenoxy)acetic acid sodium salt monohydrate, Sigma-Aldrich (D6679)

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) Agar, powder

H) 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 MS medium (medium no. 29)

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
2,4-D (0.2 mg/mL)	5 mL
Kinetin (0.2 mg/mL)	0.5 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 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 MS medium.

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

Notes

- We send LjmA 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 LjmA callus culture stably, it is essential to observe the growth of cells carefully. Because proliferation of LjmA 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 LjmA callus (about

5-mm in diameter) on 40 mL of MS medium in a 100-mL flask, and culture them for 28 days.

Appendix A: Formulation of culture medium

Table A1. Murashige and Skoog medium (medium no. 29)

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
2,4-D sodium monohydrate	1.18
Kinetin	0.1
Agar	9000