rpc00037

Lithospermum erythrorhizon M18-1 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: LS medium, 1 µM IAA, 10 µM kinetin, 1% (w/v) agar, pH 6.3 (medium no. 32) [Materials III]
- Culture conditions: 23℃, dark [Methods II]
- Subculture: 28–42-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: "*Lithospermum erythrorhizon* M18-1 cell line (rpc00037) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan."

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Introduction

Puccoon M18-1 cell line was established from a seedling of *Lithospermum erythrorhizon* Siebold & Zucc. (Mizukami *et al*. 1978, Yazaki *et al*. 2002). The M18-1 callus cells have a high capacity for producing shikonin under the induction conditions. The M18-1 cells are grown on a Linsmaier and Skoog (LS) medium supplemented with 1 μM indole-3-acetic acid (IAA) and 10 μM kinetin, and solidified with 1% (w/v) agar, pH 6.3. Our M18-1 cell culture has been maintained in the dark at 23℃ and subcultured at 28–42-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) LS_VT

Thiamine·HCl	0.16 mg/mL
myo-Inositol	40 mg/mL

D) IAA (1 mM)

IAA·K 0.213 mg/mL
Potassium 3-indoleacetate, FUJIFILM Wako Pure Chemical Corporation (#160-07531)

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) NaOH (1 N)

Glassware and equipment

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

B) Forceps, sterilized before use

Preparation of LS medium (medium no. 32)

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.

LS_VT	2.5 mL
IAA (1 mM)	1 mL
Kinetin (1 mM)	10 mL

3. Adjust the pH of the solution to 6.3 with NaOH (1 N), and fill up to 1 L with distilled water.

4. Pour 30 mL of the medium into a 100-mL flask containing 0.3 g of agar.

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

Methods

1. Pick up an appropriate amount of callus cells from a 28–42-day-old culture with a forceps and place the cells onto fresh LS medium.

2. Incubate cell cultures under the dark condition at 23℃.

Notes

- We send M18-1 cells on semi-solid LS medium in a 9-cm disposable Petri dish. The cells should be subcultured to fresh LS medium immediately after arrival.

- In order to maintain M18-1 callus culture stably, it is essential to observe the growth of cells carefully. Because proliferation of M18-1 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 seven to nine pieces of M18-1 callus (about 3–5-mm in diameter) on 30 mL of LS medium in a 100-mL flask, and culture them for 28–42 days.

References

Mizukami H, Konoshima M, Tabata M (1978) Variation in pigment production in *Lithospermum erythrorhizon* callus cultures. Phytochemistry 17: 95–97. DOI: 10.1016/S0031-9422(00)89687-9

Yazaki K, Kunihisa M, Fujisaki T, Sato F (2002) Geranyl diphosphate:4-hydroxybenzoate geranyltransferase from *Lithospermum erythrorhizon*: Cloning and characterization of a key enzyme in shikonin biosynthesis. Journal of Biological Chemistry 277: 6240–6246. DOI: 10.1074/jbc.M106387200

Appendix A: Formulation of culture medium

Table A.1. Linsmaier and Skoog medium
(medium no. 32)

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
Thiamine·HCl	0.4
myo-Inositol	100
Sucrose	30000
IAA·K	0.213
Kinetin	2.15
Agar	10000