rpc00020

Oryza sativa OS-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: AA medium, 1 mg/L 2,4-D, 0.2 mg/L kinetin, 1.2% (w/v) agar, pH 5.8 (medium no. 15) [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: "*Oryza sativa* OS-1 cell line (rpc00020) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan."

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Introduction

Rice OS-1 cell line was established from *Oryza sativa* L. (Nakasone *et al*. 1993). The OS-1 cells are grown on a AA medium supplemented with 1 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.2 mg/L kinetin, and solidified with 1.2% (w/v) agar, pH 5.8. Our OS-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) Sucrose

B) MS_micro_1

H_3BO_3	0.62 mg/mL
$MnSO_4 \cdot 5H_2O$	2.41 mg/mL
$ZnSO_4 \cdot 7H_2O$	0.86 mg/mL
KI	0.083 mg/mL
$Na_2MoO_4 \cdot 2H_2O$	0.025 mg/mL
$CuSO_4 \cdot 5H_2O$	0.0025 mg/mL
$CoCl_2 \cdot 6H_2O$	0.0025 mg/mL

C) MS_micro_2

$FeSO_4 \cdot 7H_2O$	2.78 mg/mL
Na_2-EDTA	3.73 mg/mL
Heat at 80℃ for 3–4 hours for chelating Fe	

D) AA_1

KCl	29.4 mg/mL
$CaCl_2 \cdot 2H_2O$	4.4 mg/mL
$MgSO_4 \cdot 7H_2O$	3.7 mg/mL
KH_2PO_4	1.7 mg/mL

E) AA_4

Nicotinic acid	0.05 mg/mL
Pyridoxine·HCl	0.05 mg/mL
Thiamine·HCl	0.01 mg/mL
myo-Inositol	10 mg/mL
Store at −20℃	

F) AA_5

L-Glutamine	8.77 mg/mL
L-Aspartic acid	2.66 mg/mL
L-Arginine	2.28 mg/mL
Glycine	0.75 mg/mL

Store at −20°C

G) 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)

H) 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

I) Agar, powder

J) 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 AA medium (medium no. 15)

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

   Sucrose 30 g

2. Add following stock solutions, and fill up to approximately 950 mL with distilled water.

MS_micro_1	10 mL
MS_micro_2	10 mL
AA_1	100 mL
AA_4	10 mL
AA_5	100 mL
2,4-D (0.2 mg/mL)	5 mL
Kinetin (0.2 mg/mL)	1 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 0.48 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 AA medium.

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

Notes

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

- In order to maintain OS-1 callus culture stably, it is essential to observe the growth of cells carefully. Because proliferation of OS-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 two pieces of OS-1 callus (about 10-mm in diameter) on 40 mL of AA medium in a 100-mL flask, and culture them for 28 days.

References

Nakasone S, Minami E, Imai T, Akiyama F, Ohashi Y (1993) Synchronous cell division in rice suspension cultures and cell cycle specific expression of histone H3 and PCNA genes. Bulletin of National Institute of Agrobiological Resources (Japan) 8: 1–10. (in Japanese) http://agriknowledge.affrc.go.jp/RN/2010490447

Appendix A: Formulation of culture medium

Table A1. AA medium
(medium no. 15)

Chemical	Concentration (mg/L)
KCl	2940
L-Glutamine	877
L-Aspartic acid	266
L-Arginine	228
$CaCl_2 \cdot 2H_2O$	440
$MgSO_4 \cdot 7H_2O$	370
KH_2PO_4	170
H_3BO_3	6.2
$MnSO_4 \cdot 5H_2O$	24.1
$ZnSO_4 \cdot 7H_2O$	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	75
myo-Inositol	100
Sucrose	30000
2,4-D sodium monohydrate	1.18
Kinetin	0.2
Agar	12000