rpc00100

Athyrium yokoscense AY-01 cell suspension culture

Components

- Domestic delivery: A 50-mL plastic conical centrifuge tube, containing cell suspension
- Overseas delivery: A 250-mL plastic Erlenmeyer flask, containing cells placed on semi-solid medium

Notice

- Subculture the cells to fresh medium immediately after arrival [Notes I, II].
- 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: 1/2mMS medium, 1 mg/L kinetin, pH 5.8 (medium no. 60) [Materials III]
- Culture conditions: 24℃, continuous light, 100 rpm [Methods II]
- Subculture: 14-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: "*Athyrium yokoscense* AY-01 cell line (rpc00100) 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

AY-01 cell line was established from a leaf of a metal hyper-tolerant fern *Athyrium yokoscense* (Franch. & Sav.) Christ (Yoshihara *et al*. 2005). The AY-01 cells are grown in a 1/2 modified Murashige and Skoog (1/2mMS) medium supplemented with 1 mg/L kinetin, pH 5.8. Our AY-01 cell culture has been maintained under the continuous light at 24℃ with rotary shaking at 100 rpm and subcultured at 14-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) 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) KOH (1 N)

Glassware and equipment

A) Erlenmeyer flask (300 mL), capped with two layers of aluminum foil

B) Forceps, sterilized before use

Preparation of 1/2mMS medium (medium no. 60)

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

MS salt mix	0.5 bag (equivalent with 0.5 L)
Sucrose	30 g

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

MS_VT	0.5 mL
MS_inositol	1.25 mL
Kinetin (0.2 mg/mL)	5 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 100 mL of the medium into a 300-mL flask.

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

Methods

1. Transfer seven to nine cell clumps from 14-day-old culture to 100 mL of fresh 1/2mMS medium with a forceps.

2. Incubate cell cultures on a rotary shaker at 100 rpm under the continuous light condition (photosynthetic photon flux density 60 μmol m^{-2} s^{-1}) at 24℃.

Notes

- For domestic customers: We send AY-01 cell suspension in a 50-mL disposable conical centrifuge tube. The cells should be transferred to fresh 1/2mMS medium immediately after arrival.

- For overseas customers: We send AY-01 cells placed on semi-solid 1/2mMS medium in a 250-mL disposable Erlenmeyer flask. The cells should be transferred to fresh 1/2mMS medium immediately after arrival. Transfer the cell clumps to Erlenmeyer flasks containing fresh liquid medium with a forceps.

- In order to maintain AY-01 cell suspension cultures stably, it is essential to transfer an adequate amount of cells to fresh 1/2mMS medium in every subculture. The amount of cells may vary from one lab to another, because proliferation of AY-01 cells is affected by culture conditions, such as a room temperature, rotation speed of a rotary shaker, and aeration condition of the culture.

- In order to obtain good aeration of a suspension culture, a silicone sponge plug may be used instead of the aluminum foil cap (*e.g.*, cap-type Silicosen; Shin-Etsu Polymer, Tokyo, Japan; https://www.shinpoly.co.jp/en/product/product/medical/plugs.html).

References

Yoshihara T, Tsunokawa K, Miyano Y, Arashima Y, Hodoshima H, Shoji K, Shimada H, Goto F (2005) Induction of callus from a metal hypertolerant fern, *Athyrium yokoscense*, and evaluation of its cadmium tolerance and accumulation capacity. Plant Cell Reports 23: 579–585. DOI: 10.1007/s00299-004-0877-9

Appendix A: Formulation of culture medium

Table A.1. 1/2 modified Murashige and Skoog medium (medium no. 60)

Chemical	Concentration (mg/L)
KNO_3	950
NH_4NO_3	825
$CaCl_2 \cdot 2H_2O$	220
$MgSO_4 \cdot 7H_2O$	185
KH_2PO_4	85
H_3BO_3	3.1
$MnSO_4 \cdot 4H_2O$	11.15
$ZnSO_4 \cdot 7H_2O$	4.3
KI	0.415
$Na_2MoO_4 \cdot 2H_2O$	0.125
$CuSO_4 \cdot 5H_2O$	0.0125
$CoCl_2 \cdot 6H_2O$	0.0125
$FeSO_4 \cdot 7H_2O$	13.9
Na_2-EDTA	18.65
Nicotinic acid	0.25
Pyridoxine·HCl	0.25
Thiamine·HCl	0.05
Glycine	1
myo-Inositol	50
Sucrose	30000
Kinetin	1