rpc00085

Nicotiana tabacum ATR-r 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: mLS medium, 10 μM NAA, 1 μM kinetin, pH 5.7 (medium no. 55) [Materials III]
- Culture conditions: 24℃, continuous light, 90 rpm [Methods II]
- Subculture: 21-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* ATR-r cell line (rpc00085) 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

Tobacco ATR-r cell line is a mutant cell line resistant to photosynthesis-inhibiting herbicides 2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine (atrazine) and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (Sato *et al.* 1988, Sigematsu *et al.* 1989). The ATR-r cell line was selected from a photoautotrophic NI cell line (rpc00084) that was established from a pith of *Nicotiana tabacum* L. cultivar Samsun NN (Yamada and Sato 1978). The ATR-r cells are grown in a modified Linsmaier and Skoog (mLS) medium supplemented with 10 μM 1-naphthaleneacetic acid (NAA) and 1 μM kinetin, pH 5.7. Our ATR-r cell culture has been maintained under the continuous light at 24℃ with rotary shaking at 90 rpm and subcultured at 21-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) NAA (1 mM)

NAA·K	0.224 mg/mL

Potassium 1-naphthylacetate, FUJIFILM Wako Pure Chemical Corporation (#161-04021)

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) KOH (1 N)

Glassware and equipment

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

B) Laboratory dispensing spoon (head size, 1.2 × 0.8 cm), sterilized before use

Preparation of mLS medium (medium no. 55)

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	5 mL
NAA (1 mM)	10 mL
Kinetin (1 mM)	1 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 25 mL of the medium into a 100-mL flask.

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

Methods

1. Transfer two to four spoons of cells from a 21-day-old culture into 25 mL of fresh mLS medium with a laboratory dispensing spoon.

2. Incubate cell cultures on a rotary shaker at 90 rpm under the continuous light condition (photosynthetic photon flux density 50–60 μmol m^{-2} s^{-1}) at 24℃.

Notes

- For domestic customers: We send ATR-r cell suspension in a 50-mL disposable conical centrifuge tube. The cells should be transferred to fresh mLS medium immediately after arrival.

- For overseas customers: We send ATR-r cells placed on semi-solid mLS medium in a 250-mL disposable Erlenmeyer flask. The cells should be transferred to fresh mLS medium immediately after arrival. Collect the cells from the semi-solid medium with a spatula and transfer them to Erlenmeyer flasks containing fresh liquid medium.

- In order to maintain ATR-r cell suspension cultures stably, it is essential to transfer an adequate amount of cells to fresh mLS medium in every subculture. The amount of cells may vary from one lab to another, because proliferation of ATR-r 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

Sato F, Shigematsu Y, Yamada Y (1988) Selection of an atrazine-resistant tobacco cell line having a mutant *psbA* gene. Molecular and General Genetics 214: 358–360. DOI: 10.1007/BF00337736

Sigematsu Y, Sato F, Yamada Y (1989) The mechanism of herbicide resistance in tobacco cells with a new mutation in the QB protein. Plant Physiology 89: 986–992. DOI: 10.1104/pp.89.3.986

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Appendix A: Formulation of culture medium

Table A.1. modified Linsmaier and Skoog medium (medium no. 55)

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	0.8
myo-Inositol	200
Sucrose	30000
NAA·K	2.24
Kinetin	0.215