rpc00047

Phyllostachys nigra Pn 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: mMS medium, 10 µM picloram, pH 5.7 (medium no. 40) [Materials III]
- Culture conditions: 27°C, dark, 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: "*Phyllostachys nigra* Pn cell line (rpc00047) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan."

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Introduction

Bamboo Pn cell line was established from a shoot of *Phyllostachys nigra* (Lodd. ex Lindl.) Munro var. *Henonis* (Ogita 2005). The Pn cells highly accumulate β-1,3-glucan in a cell wall. The cell line can be genetically transformed by a particle bombardment method (Ogita *et al*. 2011). The Pn cells are grown in a modified Murashige and Skoog (mMS) medium supplemented with 10 μM picloram, pH 5.7. Our Pn cell culture has been maintained in the dark at 27℃ 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) KH_2PO_4 (100 mg/mL)

KH_2PO_4	100 mg/mL

D) MS_VT

Nicotinic acid	0.5 mg/mL
Pyridoxine·HCl	0.5 mg/mL
Thiamine·HCl	0.1 mg/mL
Glycine	2 mg/mL

E) MS_inositol

myo-Inositol	40 mg/mL

F) Picloram (10 mM)

Picloram	2.415 mg/mL

Dissolve picloram in dimethyl sulfoxide

G) KOH (1 N)

Glassware

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

B) Pipette (10 mL; large tip opening) and a bulb, sterilized by autoclaving at 121℃ for 20 min

Preparation of mMS medium (medium no. 40)

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.

KH_2PO_4 (100 mg/mL)	5.1 mL
MS_VT	1 mL
MS_inositol	2.5 mL
Picloram (10 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 100 mL of the medium into a 300-mL flask.

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

Methods

1. Agitate a 14-day-old culture well and transfer 1–1.4 mL of cell suspension to 100 mL of fresh mMS medium with a pipette.

2. Incubate cell cultures on a rotary shaker at 100 rpm under the dark condition at 27℃.

Notes

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

- For overseas customers: We send Pn cells placed on semi-solid mMS medium in a 250-mL disposable Erlenmeyer flask. The cells should be transferred to fresh mMS 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 Pn cell suspension cultures stably, it is essential to transfer an adequate amount of cells to fresh mMS medium in every subculture. The amount of cells may vary from one lab to another, because proliferation of Pn 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

Ogita S (2005) Callus and cell suspension culture of bamboo plant, *Phyllostachys nigra*. Plant Biotechnology 22: 119–125. DOI: 10.5511/plantbiotechnology.22.119

Ogita S, Kikuchi N, Nomura T, Kato Y (2011) A practical protocol for particle bombardment-mediated transformation of *Phyllostachys* bamboo suspension cells. Plant Biotechnology 28: 43–50. DOI: 10.5511/plantbiotechnology.10.1101a

Appendix A: Formulation of culture medium

Table A.1. modified Murashige and Skoog medium (medium no. 40)

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	680
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
Picloram	2.415