

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 μ M 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.”

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

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 μ M picloram, pH 5.7. Our Pn cell culture has been maintained in the dark at 27°C with rotary shaking at 100 rpm and subcultured at 14-day intervals.

Materials

Chemicals and stock solutions

(All stock solutions are stored at 4°C)

A) MS salt mix

Murashige and Skoog Plant Salt Mixture, FUJIFILM Wako Pure Chemical Corporation (#392-00591)

B) Sucrose

C) KH₂PO₄ (100 mg/mL)

KH ₂ PO 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

<i>myo</i> -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°C 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 ₂ PO 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°C 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°C.

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. Transfer settled cells to Erlenmeyer flasks containing fresh liquid medium with a pipette.
- 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](https://doi.org/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](https://doi.org/10.5511/plantbiotechnology.10.1101a)

Appendix A: Formulation of culture medium

Table A1. modified Murashige and Skoog medium
(medium no. 40)

Chemical	Concentration (mg/L)
KNO ₃	1900
NH ₄ NO ₃	1650
CaCl ₂ ·2H ₂ O	440
MgSO ₄ ·7H ₂ O	370
KH ₂ PO ₄	680
H ₃ BO ₃	6.2
MnSO ₄ ·4H ₂ O	22.3
ZnSO ₄ ·7H ₂ O	8.6
KI	0.83
Na ₂ MoO ₄ ·2H ₂ O	0.25
CuSO ₄ ·5H ₂ O	0.025
CoCl ₂ ·6H ₂ O	0.025
FeSO ₄ ·7H ₂ O	27.8
Na ₂ -EDTA	37.3
Nicotinic acid	0.5
Pyridoxine·HCl	0.5
Thiamine·HCl	0.1
Glycine	2
<i>myo</i> -Inositol	100
Sucrose	30000
Picloram	2.415