rpc00087

Bruguiera sexangula BsLs 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: AA medium, 0.02 µM 2,4-D, 2 µM 4-CPPU, pH 6.2 (medium no. 57) [Materials III]
- Culture conditions: 27℃, dark, 130 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: "*Bruguiera sexangula* BsLs cell line (rpc00087) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan."

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Introduction

BsLs cell line was established from a leaf of a mangrove species *Bruguiera sexangula* (Lour.) Poir. (Kura-Hotta *et al*. 2001). The BsLs suspension cells have high salt tolerance. The BsLs cells are grown in a Amino Acid (AA) medium supplemented with 0.02 μM 2,4-dichlorophenoxyacetic acid (2,4-D) and 2 μM *N*-(2-chloro-4-pyridyl)-*N*′-phenylurea (4-CPPU), pH 6.2. Our BsLs cell culture has been maintained in the dark at 27℃ with rotary shaking at 130 rpm and subcultured at 21-day intervals.

Materials

Chemicals and stock solutions

(All stock solutions are stored at 4℃)

A) Sucrose

B) $CaCl_2$ (0.6 M)

$CaCl_2 \cdot 2H_2O$	88 mg/mL

C) AA_macro

KCl	149 mg/mL
$MgSO_4 \cdot 7H_2O$	37 mg/mL
KH_2PO_4	17 mg/mL

D) 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

E) Fe(III)-EDTA (20 mM)

Fe(III)-EDTA	8.422 mg/mL

Ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid, iron (III), sodium salt, trihydrate, FUJIFILM Wako Pure Chemical Corporation (#343-01241)

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) AA_VT

Nicotinic acid	0.05 mg/mL
Pyridoxine·HCl	0.05 mg/mL
Thiamine·HCl	0.01 mg/mL
Glycine	0.16 mg/mL
Store at −20°C	

H) 2,4-D (1 mM)

2,4-D sodium monohydrate	0.261 mg/mL

(2,4-Dichlorophenoxy)acetic acid sodium salt monohydrate, Sigma-Aldrich (D6679)

I) 4-CPPU (2 mM)

4-CPPU	0.495 mg/mL

N-(2-Chloro-4-pyridyl)-*N*′-phenylurea, Sigma-Aldrich (C2791); Dissolve 4-CPPU in ethanol and store at −20°C

J) NaOH (1 N)

Glassware

A) Erlenmeyer flask (100 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 AA medium (medium no. 57)

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.

$CaCl_2$ (0.6 M)	5 mL
AA_macro	10 mL
MS_micro_1	10 mL
Fe(III)-EDTA (20 mM)	5 mL
AA_5	100 mL
AA_VT	10 mL
2,4-D (1 mM)	0.02 mL

4-CPPU (2 mM)	1 mL

3. Adjust the pH of the solution to 6.2 with NaOH (1 N), and fill up to 1 L with distilled water.

4. Pour 20 mL of the medium into a 100-mL flask.

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

Methods

1. Agitate a 21-day-old culture well and transfer 2 mL of cell suspension to 20 mL of fresh AA medium with a pipette.

2. Incubate cell cultures on a rotary shaker at 130 rpm under the dark condition at 27℃.

Notes

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

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

Kura-Hotta M, Mimura M, Tsujimura T, Nemoto-Washitani S, Mimura T (2001) High salt treatment-induced Na^+ extrusion and low salt treatment-induced Na^+ accumulation

in suspension-cultured cells of the mangrove plant, *Bruguiera sexangula*. Plant, Cell & Envrionment 24: 1105–1112. DOI: 10.1046/j.0016-8025.2001.00761.x

Appendix A: Formulation of culture medium

Table A.1. Amino Acid medium
(medium no. 57)

Chemical	Concentration (mg/L)
KCl	1490
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
Fe(III)-EDTA	42.11
Nicotinic acid	0.5
Pyridoxine·HCl	0.5
Thiamine·HCl	0.1
Glycine	76.6
Sucrose	30000
2,4-D sodium monohydrate	0.00522
4-CPPU	0.495