Maintenance of Tobacco GT16 Cell Suspension Culture

Introduction

The Tobacco GT16 cell line is transgenic BY-2 cell lines expressing Green Fluorescent Protein (GFP) fused with tubulin α (Kumagai et al. 2001). Because microtubules can be visualized by using a fluorescence microscope, the GT16 cell line is used for studying detailed structures of plant cells. A parent cell line BY-2 was derived from a callus induced from a stem of *Nicotiana tabacum* L. cultivar Bright Yellow 2 (Nagata *et al.* 1992). Our GT16 cells have been maintained in a modified Linsmaier and Skoog (mLS) medium in dark at 27°C and subcultured at one-week intervals.

Materials

I. Stock Solution and Chemicals

A) Modified LS_VT (100 ml)

Thiamine•HCl	40 mg
myo-Inositol	4 g

B) BY2_P (100 ml)

KH_2PO_4	8 g

C) MS salt mix.
Murashige and Skoog Plant Salt Mixture, Wako Pure Chemical Industries (catalog #392-00591)

D) Sucrose

E) 0.2 mg/ml 2,4-D (100 ml)

2,4-Dichlorophenoxyacetic acid	20 mg

II. Glassware

（All are sterilized by autoclaving at 121°C for 20 min）

A) Erlenmeyer flask (300 ml), capped with two layers of aluminum foil
B) Pipette, large tip opening (10 ml), and a bulb.

III. Preparation of mLS Medium

A) Dissolve one bag (1 L) of the MS salt mixture and 30 g of sucrose in approximately 800 ml of distilled water.
B) Add following stock solutions to A), and fill up to approximately 950 ml with distilled water.

Modified LS_VT	2.5 ml
BY2_P	2.5 ml
0.2 mg/ml 2,4-D	1 ml

C) Adjust the pH of the solution to 5.8 with 0.2 N KOH, and fill up to 1 L with distilled water.
D) Pour 95 ml of mLS medium into a flask, and autoclave the flask at 121°C for 20 min.

Methods

I. Agitate a one-week-old culture well and transfer 3.0–3.8 ml of cell suspension to 95 ml of fresh mLS medium with a pipette.

II. Incubate cell cultures on a rotary shaker at 130 rpm under dark condition at 25–27°C.

Notes

I. We transport the GT16 cells on semi-solid mLS medium in 250-ml disposable flasks. The cells should be transferred to fresh mLS medium immediately after arrival. Cell suspension cultures should be established using small fractions of the original culture because some cells may have been damaged during transport. Collect the light yellow cells from the medium with a spatula and transfer them to Erlenmeyer flasks containing fresh liquid medium (*e.g.*, 20 ml of medium in a 100-ml flask). Brown cells should not be used because they are damaged.

II. To maintain the GT16 cell suspension culture stably, an adequate amount of cell suspension should be transferred to fresh mLS medium for every subculturing. The amount of cell suspension may vary from one lab to another, because proliferation of GT16 cells is affected by culture conditions, such as a room temperature, rotation speed of the rotary shaker, and aeration conditions of the culture. GT16 cell suspension culture should reach a stationary phase around 7 days after culturing. In addition, extreme dilution of GT16 cell suspension culture below 1:100 results in suppression of cell division.

III. A low growth rate of parent BY-2 cell line is sometimes caused by poor aeration of suspension cultures (Kumagai-Sano *et al.* 2007). The aluminum foil caps should be closed loosely to obtain good aeration. Silicone caps may be used instead of the aluminum foil caps.

References

Kumagai F, Yoneda A, Tomida T, Sano T, Nagata T, Hasezawa S (2001) Fate of nascent microtubules organized at the M/G1 interface, as visualized by synchronized tobacco BY-2 cells stably expressing GFP-Tubulin: time-sequence observations of the reorganization of cortical microtubules in living plant cells. Plant and Cell Physiology 42: 723-732

Kumagai-Sano F, Hayashi T, Sano T, Hasezawa S (2007) Cell cycle synchronization of tobacco BY-2 cells. Nature Protocols 1: 2621-2627

Nagata T, Nemoto Y, Hasezawa S (1992) Tobacco BY-2 cell line as the “HeLa” cell in the cell biology of higher plants. International Review of Cytology 132: 1-30

Appendix 1 Composition of modified Linsmaier and Skoog medium

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	370
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	1
myo-Inositol	100
Sucrose	30000
2,4-Dichlorophenoxyacetic acid	0.2