Maintenance of Rice OS-1 Callus Cultures

Introduction

The OS-1 is a cell line of rice *Oryza sativa* L. (Nakasone *et al.* 1986). Our OS-1 cells have been maintained on AA medium solidified with 12 g/L agar in dark at 27°C and subcultured at four-week intervals.

Materials

I. Stock Solution and Chemicals

A) Sucrose

B) MS_micro1 (1000 mL)

- H$_3$BO$_3$: 620 mg
- MnSO$_4$·4H$_2$O: 2230 mg
- ZnSO$_4$·7H$_2$O: 860 mg
- Na$_2$MoO$_4$·2H$_2$O: 25 mg
- CuSO$_4$·5H$_2$O: 2.5 mg
- CoCl$_2$·6H$_2$O: 2.5 mg
- KI: 83 mg

C) MS_micro2 (1000 mL)

- Na$_2$·EDTA: 3730 mg
- FeSO$_4$·7H$_2$O: 2780 mg

Heat at 80°C for 3-4 hours for chelating Fe.

D) AA_No1 (500 mL)

- CaCl$_2$·2H$_2$O: 2.2 g
- MgSO$_4$·7H$_2$O: 1.85 g
- KH$_2$PO$_4$: 0.85 g
- KCl: 14.7 g

E) AA_No4 (100 mL)

- myo-Inositol: 1000 mg
- Nicotinic acid: 5 mg
- Pyridoxine-HCl: 5 mg
- Thiamine-HCl: 1 mg

Store at -20°C.

F) AA_No5 (500 mL)

- Glycine: 0.375 g
- L-Glutamine: 4.385 g
- L-Aspartic acid: 1.33 g
- L-Arginine: 1.14 g

Store at -20°C.

G) 0.2 mg/mL 2,4-D (100 mL)

- 2,4-Dichlorophenoxyacetic acid: 20 mg

H) 0.2 mg/mL Kinetin (100 mL)

- Kinetin: 20 mg

I) Agar, powder
II. Glassware and Equipment

A) Erlenmeyer flask (100 ml), capped with two layers of aluminum foil
B) Forceps, sterilized before use

III. Preparation of AA Medium

A) Dissolve 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.
   - MS_micro1 10 mL
   - MS_micro2 10 mL
   - AA_No1 100 mL
   - AA_No4 10 mL
   - AA_No5 100 mL
   - 0.2 mg/mL 2,4-D 5 mL
   - 0.2 mg/mL Kinetin 1 mL
C) Adjust the pH of the solution to 5.8 with 1 N KOH, and fill up to 1 L with distilled water.
D) Pour 40 mL of AA medium into a flask containing 0.48 g of agar.
E) Autoclave the flask at 121°C for 20 min.

Methods

I. Pick up an appropriate amount of callus cells from a four-week-old culture with a forceps and place the cells onto fresh AA medium.

II. Incubate callus cultures under dark condition at 27°C.

Notes

I. We transport the OS-1 cells on semi-solid AA medium in 250-ml disposable flasks. The cells should be transferred to fresh AA medium immediately after arrival.

II. To maintain the OS-1 callus culture stably, the growth of cells should be observed carefully. Because proliferation of OS-1 cells is affected by culture conditions, such as a room temperature, aeration conditions of the culture and so on, an amount of cells transferred to fresh medium and the subculture intervals may vary from one lab to another. We usually inoculate two pieces of OS-1 callus (about 10-mm in diameter) on 40 ml of semi-solid AA medium in a 100-ml flask, and culture them for four weeks (Figure 1).

III. It is essential to use good and healthy cells for subculturing.
References

Figure 1: Rice OS-1 callus culture

OS-1 cells were cultured in a 100-ml flask containing 40 ml of semi-solid AA medium under dark condition at 27°C for 0 (A) and 4 (B) weeks.
## Appendix 1 Composition of AA medium

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration (mg/L)</th>
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<tbody>
<tr>
<td>KCl</td>
<td>2940</td>
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<tr>
<td>CaCl₂·2H₂O</td>
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<tr>
<td>MgSO₄·7H₂O</td>
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<tr>
<td>KH₂PO₄</td>
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<tr>
<td>H₃BO₃</td>
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<tr>
<td>MnSO₄·4H₂O</td>
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<td>ZnSO₄·7H₂O</td>
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<tr>
<td>KI</td>
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<td>Na₂MoO₄·2H₂O</td>
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<tr>
<td>CuSO₄·5H₂O</td>
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<tr>
<td>CoCl₂·6H₂O</td>
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<td>FeSO₄·7H₂O</td>
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<td>Na₂·EDTA</td>
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<tr>
<td>Nicotinic acid</td>
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<td>Kinetin</td>
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<tr>
<td>Agar</td>
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