III. MEDIA

◎ In the KU-MACC, all of the strains are cultured in Provasoli' s enriched sea water (PES or PESI) or ASP_{12}.

1. Preparation of PES and PESI medium

(1) Collect un-polluted (preferably offshore) sea water and remove particulate matter with filters
(2) Sterilize the filtered sea water by autoclaving at 121° C for 20 min.
(3) Add 20 ml of PES or PESI medium stock solution to 1000 ml of the sterilized sea water.

Preparation of PES stock solution
i) Dissolve appropriate weighed quantities (APPENDIX) of Tris, $NaNO_3$, Na_2-glycerophosphate, Fe stock solution, P-2 metal mix, Vitamin B_{12} stock solution, Thiamine-HCl stock solution, and Biotin stock solution, into 300 ml of distilled water.
ii) Adjust to pH 7.8 with 1N HCl or NaOH, and then dilute to 1000 ml final volume with distilled water.
iii) Dispense 20 ml of the medium into sealed tubes and sterilize the medium by autoclaving at 121° C for 20 min, and store at 4° C.

Preparation of PESI stock solution
i) Dissolve appropriate weighed quantities (APPENDIX) of Tris, $NaNO_3$, Na_2-glycerophosphate, Fe stock solution, P-2 metal mix, KI stock solution into 300 ml of distilled water.
ii) Adjust to pH 7.8 with 1N HCl or NaOH, and then dilute to 1000 ml of final volume with distilled water.
iii) Dispense 20 ml of the solution into sealed tubes and sterilize by autoclaving at 121° C for 20 min, and store at 4° C.

Preparation of Fe stock solution
i) Dissolve appropriate weighed quantities (APPENDIX), in this order, of Na_2-EDTA · $2H_2O$, and $Fe(NH_4)_2(SO_4)_2$ · $6H_2O$ into about 450 ml of distilled water.
ii) Dilute to 500 ml final volume with distilled water, and store at 4° C.

Preparation of P-2 metal mix stock solution
i) Dissolve appropriate weighed quantities (APPENDIX), in this order, of Na_2-EDTA · $2H_2O$, H_3BO_3, $FeCl_3$ · $6H_2O$, $MnSO_4$ · $4H_2O$, $CoSO_4$ · $7H_2O$, and $ZnSO_4$ · $7H_2O$ into about 450 ml of distilled water.
ii) Dilute to 500 ml final volume with distilled water, and store at 4° C.

References:
Andersen, R.A. (2005) Algal culturing techniques. Elsevier Academic Press, San Diego, California. In: Kawai, H., Motomura, T. and Okuda, K. Chapter 9, pp. 133-143.
Provasoli, L. 1966. Media and prospects for the cultivation of marine algae. In: Watanabe, A. and Hattori, A. [Eds] Cultures and Collections of algae. Proceedings of the US-Japan conference held at Hakone, 12-15 Sept. 1966. Jpn. Soc. Plant., Physiol., pp. 63-75

2. Preparation of ASP_{12}.

i) Dissolve appropriate weighed quantities (APPENDIX) of reagents into about 900 ml of distilled water.
ii) Adjust to pH 7.8 with 1N HCl or NaOH, and then dilute to 1000 ml of final volume with distilled water.
iii) Sterilize the medium by autoclaving at 121° C for 20 min, and store at 4° C

References: Chihara, M. and Nishizawa, K. (1979) Techniques of Algal studying. Kyoritsu Press, Tokyo, pp. 287-288

IV. LIST OF STRAINS

1. Notes on data of strains

◎ The strains are listed by class in alphabetical order. Strains with the same scientific name are arranged in order of their strain number. A detailed example of a strain description is presented below.

Schizocladia ischiensis E.C. Henry, Okuda et Kawai[1)]

Strain: KU-333[2)]. **Origin:** Ischia Isl., Naples, Italy (1987-10-21)[3)]. Leg./Isol. E. Henry[4)]. **Culture:** PES; 10°C[5)]. **Note:** Type culture; 18S rDNA, *rbc*L[6)]. **Reference:** Kawai et al. (2003)[7)]

1) Scientific name with authority
2) KU strain code (used with the initials "KU-").
3) Collection locality and collection data in parentheses
4) Lego/Isolator
5) Culture medium and culture conditions (temperature)
6) Information of a strain (generation, sex, type culture, etc.), DNA sequencing region used for identification, State of cryopreservation (If strains are cryopreserved, shown as "cryopreserved"). In case of a strain deposited from Hokkaido University, the original code of Hokkaido University is described at the head of the line (e.g., [HOK-123]).
7) References (which are lists of studies performed using this strain, and their details are described in p. 72-74)