Maintenance of Cultures

Cultures sent to customers remain alive for at least 14 days provided that the following precautions are observed. Cultures should be unpacked immediately after receipt and stored at 15-18°C under low light intensity (north window, no direct sun light, or weak white fluorescent light). Screw caps or vessels should be loosened but not removed. Further maintenance or multiplication of cultures requires transfer into new culture media. This presupposes experience in simple microbial techniques.

Many species are cultivated and dispatched on agar media for safety reasons but develop their morphological characteristics only in liquid media, e.g. flagellates, colony-forming Volvocales and Chlorococcales. For teaching purposes these species should be transferred into liquid media 2-3 weeks before demonstration, e.g. into Soil Water Media, Basal Medium, or Desmidiacean Medium.

Culture Media

The following media have proved suitable for the maintenance of cultures in test tubes at the SAG for many years. The recipes originate from E. G. Pringsheim and W. Koch, unless stated otherwise. It must be emphasized that the maintenance medium indicated is not always the best medium for the cultivation of a species. There are other media which are just as suitable, e.g. those given in the catalogues of other culture collections of algae (Thompson et al., 1988, Watanabe and Nozaki, 1994, Andersen et al., 1991, Rippka and Herdman, 1992, Starr and Zeikus, 1993). Mass algal culture often requires more concentrated media (for recipes and methods consult Kuhl and Lorenzen, 1964; Starr, 1971; Stein, 1973; Guillard, 1975; Werner, 1982; Castenholz, 1988; Richmond, 2004; Andersen, 2005).

All solutions should be made up with de-ionized water. Media are usually prepared from stock solutions of macronutrients, trace metals, and vitamins which are added to a large proportion of the final volume of water in order to avoid precipitation.

Media may be used as liquid or solidified by 1.0-1.5% agar. Before sterilization the agar has to be dissolved in the medium in a steamer. After this test tubes should be filled with 10 ml of the hot medium, closed with cotton plugs, sterilized (usually by autoclaving at 121°C for 15 min.) and may be stored for several weeks, after cooling, in a refrigerator. Solid media for Cyanobacteria are prepared by mixing, after cooling to 50°C, equal volumes of separately autoclaved double strength solutions of the mineral salts medium and either agar to give a final agar concentration of 0.6-1.0 %.

1. Basal Medium (= ES "Erddekokt + Salze")

	stock solution [g/100 ml]	nutrient solution [ml]
KNO_3	1	20
K_2HPO_4	0.1	20
$MgSO_4 . 7H_2O$	0.1	20
soil extract *		30
micronutrient solution **		5
de-ionized or distilled water		905

* Preparation of soil extract: Fill a 6 litre flask one third with garden or leaf soil of medium, but not too great humus content which does not contain fertilizers or plant protective agents. Success of soil extract depends on selection of suitable soils. Those with high clay content are usually less satisfactory. Add de-ionized water until it stands 5 cm above the soil and sterilize by heating in a steamer for one hour twice in a 24 h interval. Separate the decanted extract from particles by centrifugation. Fill into small containers of stock solution each of a size appropriate to making a batch of media, autoclave for 20 min at 121°C and store in the refrigerator.

** Preparation of the micronutrient solution:

	stock solution [g/100 ml]	applied solution
$ZnSO_4 . 7H_2O$	0.1	1 ml
$MnSO_4 . 4H_2O$	0.1	2 ml
H_3BO_3	0.2	5 ml
$Co(NO_3)2 . 6H_2O$	0.02	5 ml
$Na_2 MoO_4 .2H_2O$	0.02	5 ml
$CuSO_4 . 5H_2O$	0.0005	1 ml
de-ionized or distilled water		981 ml
$FeSO_4 . 7H_2O$		0.7 g
EDTA (Titriplex III, Merck)		0.8 g

Autoclave the components separately in two solutions which are united after cooling.

Solution I: 881 ml distilled water + stock solutions of salts without $FeSO_4$ + 0.4 g EDTA
Solution II: 100 ml distilled water + 0.7 g $FeSO_4$ + 0.4 g EDTA
The following modifications of the Basal Medium proved suitable for many strains:

a) Basal Medium with Beef Extract (= ESFl. "Erddekokt + Salze + Fleisch"**):** Basal Medium (Medium 1) with 0.1 % beef extract.
b) Basal Medium with Peptone (= ESP "Erddekokt + Salze +Peptone"**):**
Basal Medium (Medium 1) with 0.1% proteose-peptone.
c) Basal Medium with 10 % Euglena Medium and Vitamins (= +V "Erddekokt + Salze + Euglena gracilis Medium + Vitamine"**):**
Basal Medium (Medium 1) plus 10 % Euglena Medium (medium 9) and the vitamins B_1 (5 x 10-4 g/l) and and B_{12} (5 x 10-6 g/l), added in sterile solution after autoclaving.
d) Acidified Basal Medium (= ES + H_2SO_4):
Basal Medium (Medium 1) plus 1% conc. H_2SO_4.

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