M E D I A

NAPROSTO BOŽÍ PŘEHLED MEDIÍ S PODROBNÝMI POPISY
http://www.ccap.ac.uk/media/recipes.htm#

TAKÉ ZDE

http://ccmp.bigelow.org/future/hmenuz.php?daurl=http://ccmp.bigelow.org/future/mediarecipes.html

A TAKY ZDE
http://www.epsag.uni-goettingen.de/html/culturemedia.html#listofmedia

A. Conditions for maintenance of cyanophytes and algae and nutrient solutions

Strains are kept in test tubes or in Erlanmayer flasks (100-300ml) placed on shelves, by a vertical panels of fluorescent tubes; they gives cca 1 W.m-2 (220 1x). The panel is provided with a few small incandescent bulbs to provide some infrared wavelengths. Light is controlled to 12h/12h light/dark, to save cooling system and electricity. Strains are transferred to fresh slants in cca 3-4 months intervals. After inoculation a strain is allowed to grow up in better conditions, room temperature and cca 10 W.m-2.

Note: Any nutrient solution can be used as liquid, or solidified by 1.5% of agar. In this case medium is labeled by "agar".

No.1. = ***Medium Z,*** ZEHNDER in STAUB (1961): for most of cyanophytes

Into ca 750 ml distilled water add 10 ml of individual macroelement stock solutions, 10 ml of Fe-EDTA, 0,08 ml of Gaffron's microelements, and refill with distilled water to 1000 ml.

Makroelements		Gaffron's microelements (100 ml)			
NaNO3	46,7 g/L	NiSO4(NH4)2SO4.6H2O	19,8 mg	KBr	11,9 mg
Ca(NO3)2.6H2O	5,9 g/L	V2O4(SO4)3.16H2O	3,1 mg	H3BO3	31 mg
K2HPO4	3,1 g/L	(NH4)6Mo7O24.4H2O	8,8 mg	MnSO4.4H2O	223 mg
MgSO4.7H2O	2,5 g/L	ZnSO4.7H2O	28,7 mg	Cr(NO3)3.7H2O	3,70 mg
Na2CO3	2,1 g/L	Cd(NO3)2.4H2O	15,4 mg	Co(NO3)2.H2O	14,6 mg
		Al2(SO4)3K2SO4.24H2O	47,4 mg	KJ	8,3 mg

		Na2WO4.2H2O	3,3 mg	CuSO4.5H2O	12,5 mg
Fe-EDTA (500 ml)					
0,138mg FeCl3.6H2O in 5ml 0,1N HCl + 0,186mg Chelaton III in 5ml 0,1N HCl					

No.2. = ***Medium BB*** *(Bristol modif. Bold),* BOLD (1949): for most of algae

Mix 10 ml of sol. "a", 1 ml "b", 1 ml "c", 1 ml "d", 1 ml "e" and refill with destilled water into 1 000 ml.

Stock sol."a" (1000ml)		Stock sol."b" (100ml)		Stock sol."e" (100ml)	
NaNO3	25g	Chelaton III	5,0g	ZnSO4.7H2O	0,882g
CaCl2.2H2O	2,5g	KOH	3,1g	MnCl2.4H2O	0,144g
K2HPO4	7,5g	Stock sol."c" (100ml)		Na2MoO4.2H2O	0,242g
KH2PO4	17,5g	FeSO4.7H2O	0,498g	or MoO3	0,071g
MgSO4.7H2O	7,5g	H2SO4 conc.	0,1ml	CuSO4.5H2O	0,157g
NaCl	2,5g	Stock sol."d" (100ml)		Co(NO3)2.6H2O	0,049g
		H3BO3	1,142g		

No.3. = ***Dunaliella medium,*** SCHLÖSSER (1982):

Mix 20 ml of the stock macroelements solution with 910 ml of artificial seawater and 30 ml of seawater soil extract.

Makroelements (100 ml)		Artificial seawater (1000 ml)	
KNO3	1,0 g	NaCl	60 g
K2HPO4	0,1 g	MgSO4.7H2O	10 g
MgSO4.7H2O	0,1 g	KCl	1,5 g
		CaSO4	2,0 g

Seawater soil extract: 1/3 of 6 litre flask fill with garden or leaf soil (not too great humus or clay content), add artificial seawater until it stands 5cm above the soil. Sterilize by heating in a steamer one hour, twice in a 24h interval. Separate the decanted ext ract by centrifugation. Fill into small containers, autoclave 20 minutes/1 atmosphere; store in refrigerator.

No.4. = ***Euglena medium,*** SCHLÖSSER (1982):

Mix 960 ml distilled water and 10 ml of the following individual stock solutions.

Stock solutions				
Na-acetate	10 %		Bacto-tryptone	10 %
Beef extract	10 %		Yeast extract	10 %

No.5. = ***GORHAM'S solution,*** KOEMAN & HOEK (1980):

In 1000 ml distilled water dissolve the following chemicals and add 2 ml of soil extract .

NaNO3	160 mg	CaCl2	35 mg	Chelaton III	600 mg
K2HPO4	17,5 mg	Na2SiO3	11,63 mg	Na2MoO4.2H2O	0,00705 mg
TRIS	200 mg	Na2CO3	20 mg	ZnSO4.7H2O	0,0392 mg
MgSO4.7H2O	153,6 mg	Fe3+-citrate	3 mg	CoCl2	0,0183 mg
CuSO4.5H2O	0,0156 mg	Citric acid	3 mg	MnCl2.4H2O	0,283 mg

Soil extract: 1/3 of 6 litre flask fill with garden or leaf soil (not too great humus or clay content), and deioinized water until it stands 5cm above the soil. Sterilize by heating in a steamer one hour, twice in a 24h interval. Separate the decanted ext ract by centrifugation. Fill into small containers, autoclave 20 minutes/1 atmosphere; store in refrigerator.

No.6. = ***Hydrodictyon medium,*** POCOCK (1960):

In 2700 ml distilled water dissolve the following chemicals and add 300 ml of soil extract (pH=7-7,3).

KNO3	50 mg	ZnSO4.7H2O	50 mg	Ca(NO3)2	200mg
K2HPO4	50 mg	K2CO3	70 mg	FeCl3	10 mg

Soil extract: see above.

No.7. = ***Porphyridium medium***, BRODY & EMERSON (1959): modif. PEKÁRKOVÁ (p.c.)

Mix Solution 1 with 0,5 ml of Solution 2 and 0,5 ml of Solution 3 and autoclave.

Solution 1 (1000 ml)		Solution 2 (250 ml)	
KCl	4,00g	Fe-EDTA	4,6 g
NaCl	3,13g	Solution 3 (1000 ml)	

KNO3	1,24g	H3BO3	3,09 mg
MgSO4	2,50g	MnSO4.4H2O	1,20 mg
K2HPO4	0,66g	CoSO4.7H2O	1,40 mg
Ca(NO3)2	0,17g	CuSO4.5H2O	1,24 mg
KJ	0,05g	ZnSO4.7H2O	1,43 mg
KBr	0,05g	(NH4)6Mo7O24.4H2O	1,84 mg

No.8. = ***Spirulina medium,*** SCHLÖSSER (1982):

Autoclave Solution 1 and Solution 2 separately, mix after cooling, add 5 ml of sterile Microelements solution and 6g of vitamin B12.

Solution 1 (500 ml)		Microelements solution		
NaHCO3	13,61g	dist. H2O		981ml
Na2CO3	4,03 g	ZnSO4.7H2O	0.1% sol.	1.0ml
K2HPO4	0,50 g	MnSO4.4H2O	0.1% sol.	2 ml
Solution 2 (500 ml)		H3BO3	0.2% sol.	5 ml
NaNO3	2,50g	Co(NO3)2.6H2O	0.02% sol.	5 ml
K2SO4	1,00g	Na2MoO4.2H2O	0.02% sol.	5 ml
NaCl	1,00g	CuSO4.5H2O	0.0005% sol.	1 ml
MgSO4.7H2O	0,20g	FeSO4.7H2O		0,7g
CaCl2.2H2O	0,04g	Chelaton III		0,8g
FeSO4.7H2O	0,01g			
Chelaton III	0,08g			

B. Conditions for maintenance of mosses, liverworts and ferns and nutrient solutions

Liverworts, ferns and duckweeds are maintained in 250 ml Erlenmayer's flasks on agar medium in the air conditioned room at 15oC. They are irradiated by fluorescent tubes (white) with regime 8h light/16hdark.

Medium M = *for* ***mosses*** *and* Medium JKL = *for* ***liverworts*** *and* ***ferns***, BASLEROVÁ & DVOŘÁKOVÁ (1962):

Chemicals	Stock sollutions	M (1000 ml)	JKL (1000 ml)
NH4NO3	1,0 M	1,5 ml	2,50 ml
KH2PO4	1,0 M	0,5 ml	0,75 ml
MgSO4	1,0 M	0,5 ml	0,40 ml
CaCl2	1,0 M	0,2 ml	1,00 ml
FeCl2	0,1 M	0,2 ml	0,16 ml

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
distilled water		905

* Preparation of soil extract: Fill a 6 liter 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 deionized 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
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**: Medium 1 with 0.1 % beef extract. (= ESFl. "Erddekokt + Salze + Fleisch)

b) **Basal Medium with Peptone**: Medium 1 with 0.1% proteose-peptone. (= ESP "Erddekokt + Salze +Peptone")

c) **Basal Medium with 10 % Euglena Medium and Vitamins**: 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. (= +V "Erddekokt + Salze + Euglena gracilis Medium + Vitamine)

d) Acidified Basal Medium (= ES + H_2SO_4): Medium 1 plus 1% conc. H_2SO_4.

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2. Spirulina Medium (=Spir.)

(modified from S. Aiba and T. Ogawa 1977)

Solution I:	
$NaHCO_3$	13.61 g
Na_2CO_3	4.03 g
K_2HPO_4	0.50 g
distilled water	500.0 ml

Solution II:	
$NaNO_3$	2.50 g
K_2SO_4	1.00 g
NaCl	1.00 g
$MgSO_4 . 7H_2O$	0.20 g
$CaCl_2 . 2H_2O$	0.04 g
$FeSO_4 . 7H_2O$	0.01 g
EDTA (Titriplex III, Merck)	0.08 g
micronutient solution (see medium 1)	5.0 ml
distilled water	500.0 ml

Autoclave solutions I and II separately, unite after cooling and add 5 x 10^{-6} g/l vitamin B_{12} in sterile solution, if required.

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3. Soil Water Media

These media have great advantages for may purposes so long as axenic culture is not required, e.g. for the cultivation of species of which nutrient requirements are not known, or to obtain morphologically normal growth.

Preparation: Place 1-2 cm of garden soil (E = garden soil. Modified M = loamy soil; T = peat; S = sand) in the bottom of a test tube (or bottle). The appropriate quality of soil is indicated under medium 1, preparation of soil extract. Fill up with distilled or deionized water until the container is 3/4 full and cover with cotton plug. Steam (not autoclave) for 1 h on two consecutive days.

A variety of modifications can be made adding additional materials and using different types of soil. The following have proved especially suitable:

a) Soil Water Medium with $CaCO_3$ (=CaE "Calcium + Erdwasser")

A small pinch of powdered $CaCO_3$ is placed in the bottom of the tube before soil is added.

b) Soil Water Medium with NH_4MgPO_4 (=NH4E)

A small pinch of NH_4MgPO_4 is placed in the bottom of the tube before soil is added.

c) Soil Water Medium with Pea (= ErbsE)

1/8 of a garden pea, soaked 12 h before, is placed in the bottom of the tube before soil is added.

d) Soil Water Medium with Barley (=GerstE)

1/2 of a barley corn previously soaked for 12 h, is placed in the bottom of the tube before soil is added.

e) Soil Water Medium with Wheat (=WeizenE)

1/2 of a wheat corn previously soaked for 12 h, is placed in the bottom of the tube before soil is added.

f) Soil Water Medium with Pea (= ErbsMS)

1/8 of a garden pea, soaked 12 h before, is placed in the bottom of the tube before soil (loamy soil (M) and

g) Soil Water Medium with Pea (= ErbsS)

1/8 of a garden pea, soaked 12 h before, is placed in the bottom of the tube before soil (sand, S) is added.

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4. Beggiatoa Medium (=B)

		stock solution [g/100 ml]	nutrient solution [ml]
NH_4Cl		0.4	20
K_2HPO_4		0.1	10
$CaSO_4$	saturated solution		20
$MgSO_4 . 7H_2O$		0.1	10

Na-acetate	1	10
asparagine	1	50
micronutrient solution (see medium 1)		5
distilled water		875

Sterilize for 1 h in a steamer or autoclave if axenic culture is desired. Autoclave soil extract separately and unite after cooling. Add vitamin B_{12} (5×10^{-6}g) in sterile solution.

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5. Seawater Medium (= SWES "Seewasser + Erddekokt + Salze")

	stock solution [g/100 ml]	nutrient solution [ml]
KNO_3	1	20
K_2HPO_4	0.1	20
$MgSO_4 \cdot 7H_2O$	0.1	20
soil extract (see medium 1)		30
micronutrient solution (see medium 1)		5
filtered seawater		905

Sterilize for 1 h in a steamer or autoclave if axenic culture is desired. Autoclave soil extract separately and unite after cooling. Add vitamin B_{12} (5×10^{-6}g) in sterile solution.

a) Seawater Medium with Na_2SiO_3 (= SWES + Na_2SiO_3)

Add 50 ml of a saturated solution of $Na_2SiO_3 \times 5\ H_2O$ to medium 5.

b) Seawater Medium with Selenite (= SWES + Na_2SeO_3)

Add 0.25 ml stock solution **N**a_2SeO_3 (2.36 mg / 500 ml) to 100 ml of medium 5.

Name: BG11 (Blue-Green Medium)

Description: Freshwater algae and protozoa

Stock per litre

(1) $NaNO_3$ 15.0 g

Stocks per 500 ml

(2) K_2HPO_4 2.0 g
(3) $MgSO_4.7H_2O$ 3.75 g
(4) $CaCl_2.2H_2O$ 1.80 g
(5) Citric acid 0.30 g
(6) Ammonium ferric citrate green 0.30 g
(7) $EDTANa_2$ 0.05 g
(8) Na_2CO_3 1.00 g
(9) Trace metal solution per litre
H_3BO_3 2.86 g
$MnCl_2.4H_2O$ 1.81 g
$ZnSO_4.7H_2O$ 0.22 g
$Na_2MoO_4.2H_2O$ 0.39 g
$CuSO_4.5H_2O$ 0.08 g
$Co(NO_3)_2.6H_2O$ 0.05 g

Medium

Stock solution **1** 100.0 ml
Stock solutions **2** - **8** 10.0 ml each
Stock solution **9** 1.0 ml

Method

Make up to 1 litre with deionized water. Adjust pH to 7.1 with 1M NaOH or HCl. For agar add 15.0 g per litre of *Bacteriological Agar (Oxoid L11). Autoclave at 15 psi for 15 minutes.

Supply * Unipath Ltd, Wade Road, Basingstoke, Hants RG24 0PW, UK

Ref Stanier, R.Y., Kunisawa, R., Mandel, M. & Cohen-Bazire, G. (1971) Purification and properties of unicellular blue-green algae (Order Chroococcales).
Bacteriol. Rev. 35: 171-205

Name: EG (Euglena Gracilis Medium)

Description: Freshwater algae and protozoa

Stock per litre

(1) CaCl2 stock solution

CaCl2 1.0 g

Medium per litre

Sodium acetate trihydrate 1.0 g

*"Lab-Lemco" powder (Oxoid L29) 1.0 g

*Tryptone (Oxoid L42) 2.0 g

*Yeast extract (Oxoid L21) 2.0 g

CaCl2 stock solution **(1)** 10.0 ml

Method

Add constituents above and make up to 1 litre with deionized water. For agar add 15 g per litre *Bacteriological Agar (Oxoid L11). Autoclave at 15 psi for 15 minutes.

Supply * Unipath Ltd, Wade Road, Basingstoke, Hants RG24 0PW, UK

Name: SE (Soil Extract)

Description: Constituent of several CCAP media

Preparing the soil

Site selection for a good soil is very important and for most purposes a soil from undisturbed deciduous woodland is best. Sites to avoid are those showing obvious signs of man's activity and particular care should be taken to avoid areas where fertilizers, crop sprays or other toxic chemicals may have been used.

A rich loam with good crumb structure should be sought. Stones, roots and larger invertebrates should be removed during an initial sieving through a 1 cm mesh. The sieved soil should be spread to air dry and hand picked for smaller invertebrates and roots. It should be turned periodically and picked over again. When dry it may be sieved through a finer mesh (2-4 mm) or stored as it is prior to use.

Methods

Two slightly different preparations are used at the CCAP, as follows:

SE1 (Soil Extract 1)

Used in media for marine algae

Soil is prepared as above. Air-dried soil and twice its volume of supernatant distilled water are autoclaved together at 15 psi for 2 hours and left to cool. The supernatant is then decanted and filtered through Whatman No 1 filter paper, then distributed to containers in volumes suitable for making up batches of media. The aliquots and their containers are autoclaved for an appropriate length of time (eg 1 litre or less for 15 minutes) and are then kept in a cool place (eg a refrigerator) until required.

SE2 (Soil Extract 2)

Used in media for freshwater and terrestrial protozoa

Soil is prepared as above. 105 g of air-dried sieved soil and 660 ml of deionized water are placed in a 1 litre bottle and autoclaved once at 15 psi for 15 minutes, then again after 24 hours. The contents of the bottle are left to settle (usually for at least a week) and then the supernatant is decanted and filtered. The final pH should be 7.0 - 8.0.

Notes for preparing the culture media

All solutions should be made up with **glass distilled water**. Media are usually prepared from **st**
solutions of macronutrients, trace metals, and vitamins which are added to a large proportion o
volume of water in order to avoid precipitation.
Media may be used as liquid or solidified by 1.0-1.5 % agar. In the list of media below, an **"Ag"**

that the strain is cultivated on an agarized medium in the SAG culture collection. For axen cultures, agarized media are preferred over liquid-ones because with handling of agarized media transfer it is easier to minimize contamination risks. Before sterilization the agar has to be dissol medium in a steamer. Test tubes should be filled with approx. 10 ml of the hot medium, closed a autoclaved at 121° C for 15 min. They may be stored for several weeks, after cooling, in a refrig Solid media for **cyanobacteria**: autoclave agar dissolved in water and a double strength solution salts **separately**, then mix both solutions after cooling to 50° C to give a final agar concentratio 1.0 %.

Note on biphasic soil-water media

Biphasic soil water-media may be **advantageous for healthy growth over long periods of** non-axenic strains, in particular for filamentous green algae and euglenoids. The **addition of s extract often helps to achieve the typical morphology best** (e.g. in coccoid green algae) the cells on the same medium without soil extract tend to accumulate starch or oil droplets. So strains (e.g. some colorless euglenoids) cannot be grown in defined media, but only in soil-wat Important criteria for the selection of soil include that it must not contain articifial or natural m and dung, just old garden soil with a little sand, soil from mature compost or decayed leafs is w suited. The **limitation of soil-water media** is that they are not suited to maintain axenic cult because they cannot be autoclaved. Instead, soil and water are only cooked in a steam pot re times so that bacteria which are needed to supply the water phase with nutrients by their slow organic compounds in the soil can survive. In biphasic soil-water media the growth of bacteria promoted by the addition of organic matter, e.g. one eightth of a pea or half a wheat corn, to a constant nutrient supply.