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Terry W. Hill
Rhodes College

Etta Kafer
Simon Fraser University

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Abstract

Simplified protocols for ingredients of *Aspergillus* minimal medium (MM) are presented. These either do not change the final composition of MM or at most involve only minor modifications which, in extensive comparative tests, have not shown any effects on growth patterns of all strains/cultures tested.

Improved protocols for Aspergillus minimal medium: trace element and minimal medium salt stock solutions

Terry W. Hill[1] and Etta Kafer[2]. [1]Department of Biology, Rhodes College, Memphis, TN 38112 USA. [2]Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, B.C., V5A 1S6, Canada.

Simplified protocols for ingredients of *Aspergillus* minimal medium (MM) are presented. These either do not change the final composition of MM or at most involve only minor modifications which, in extensive comparative tests, have not shown any effects on growth patterns of all strains/cultures tested.

I. Improved preparation of Hutner=s trace element (TE) solution, three versions:

A. Procedure for Hutner=s TE with full solubility at room temperature and during storage.

By first dissolving the Fe-salt and adjusting its pH separately, as follows, there is no need for heating, and occasional problems with precipitation[a] are avoided.

To prepare 200 ml of the 1000x TE solution (i.e., 1 ml required for 1 liter of MM) the Astandard@ ingredients and proportions are used, as listed below [from Scott and Kafer 1982[b] de Serres and Hollaender (eds.) Chemical Mutagens vol. 7 (Plenum) 447-479].

1. Dissolve the listed salts in 80 ml of distilled water[c] in the order indicated:

$FeSO_4 \cdot 7H_2O$ (Ferrous sulphate)	1.0	g
EDTA	10.0	g

Adjust pH upwards with KOH pellets. A golden yellow solution results above around pH 5.5, and this is sufficient to proceed.

2. Dissolve the listed salts in 80 ml of distilled water in the order indicated:

$ZnSO_4 \cdot 7H_2O$ (Zinc sulphate)	4.4	g
H_3BO_3 (Boric acid)	2.2	g
$MnCl_2 \cdot 4H_2O$ (Manganous chloride)	1.0	g
$CoCl_2 \cdot 6H_2O$ (Cobaltous chloride)	0.32	g
$CuSO_4 \cdot 5H_2O$ (Cupric sulphate)	0.32	g
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (Ammonium molybdate)	0.22	g

Combine Solutions (1) and (2), and readjust the pH to 6.5 using first KOH pellets, then KOH solutions of decreasing concentration. Bring the final volume to 200 ml with distilled water, and store at 4-8°C.

As with traditionally prepared Hutner=s TE, this solution is initially bright green, turning purple upon storage. Precipitates are never formed.

[a] In general, precipitation, if it happens to occur in Hutner=s TE, will not affect growth, provided noticeable mismeasurement is avoided. In fact, at least for *Chlamydomonas*, it is rumoured to work better if a precipitate has formed

[b] Alternate protocols for trace element solutions and MM, especially types more suitable for tests of N-metabolism mutants, are presented in this reference (e.g., Scott and Alderson, 1972, Radiat. Bot. **12**:45-50)

[c] In all protocols, distilled water can be replaced by nanopure water.

B. Modified Hutner=s TE solution: pH established with tetra-sodium-EDTA.

This method is simple and easy, and TE will contain the standard concentrations of all critical nutrients. However, there are two differences compared to standard Hutner=s TE: namely, the Na^+/K^+ balance is changed in favor of Na^+, and the molarity of EDTA is reduced. (The latter can be corrected, however; see protocol C below.)

For 100 ml, dissolve the listed salts in 80 ml of distilled water in the order indicated; most salts will dissolve easily at room temperature, and after addition of Na_4EDTA, generally no precipitation is observed.

$ZnSO_4 \cdot 7H_2O$ (Zinc sulphate)	2.2	g
H_3BO_3 (Boric acid)	1.1	g
$MnCl_2 \cdot 4H_2O$ (Manganous chloride)	0.5	g
$FeSO_4 \cdot 7H_2O$ (Ferrous sulphate)	0.5	g
$CoCl_2 \cdot 6H_2O$ (Cobaltous chloride)	0.16	g
$CuSO_4 \cdot 5H_2O$ (Cupric sulphate)	0.16	g
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (Ammonium molybdate)	0.11	g
$Na_4EDTA \cdot 4H_2O$ (EDTA, tetrasodium salt)	6.0	g

Bring the final volume to 100 ml with distilled water, autoclave, and store at 4-8EC. The unadjusted pH will be about 6.5.

C. Modified Hutner=s TE solution with standard EDTA molarity: pH established with mixed sodium-EDTA salts.

Dissolve salts as in the preceding protocol, but substitute the following combination of sodium-EDTA salts:

$Na_4EDTA \cdot 4H_2O$ (EDTA, tetrasodium salt)	6.5	g
$Na_2EDTA \cdot 2H_2O$ (EDTA, disodium salt)	0.77	g

The pH will be somewhat higher than 6.5 (around 7.0 B 7.2, varying with the temperature when measured) but disodium EDTA will dissolve more easily at this less acid pH [since A$Na_2EDTA \cdot 2H_2O$ does not dissolve well at pH < 8.0@ (Sambrooke et al., 1989, vol. 3, p. B11)]. However when Minimal Medium is prepared the final pH will be at the desired level of 6.5-6.6 (see II B below).

II. Improved Preparation of Minimal Medium (MM): Salt stock solutions and MM.

A. Minimal Medium Salts, 2 stock solutions

1. Salt mix Alacking $MgSO_4$@ (20x) Stock[d]: use 50 ml for 1 liter MM

For 1000 ml, dissolve the listed salts in 800 ml of distilled water in the order indicated.

$NaNO_3$ (Sodium nitrate)	120.0	g
KCl (Potassium chloride)	10.4	g
KH_2PO_4 (Potassium phosphate, monobasic)	16.3	g
K_2HPO_4 (Potassium phosphate, dibasic)	20.9	g

Bring the final volume to 1000 ml with distilled water. Store at 4-8EC or autoclave and keep at room temperature.

2. $MgSO_4$ Solution (200x) Stock[d]: use 5 ml for one liter of MM.

For 100 ml, dissolve in 80 ml of distilled water:

$MgSO_4 \cdot 7H_2O$ (Magnesium sulphate)	10.4	g

Bring the final volume to 100 ml with distilled water and autoclave; once opened, store at 4-8°C.

B. Minimal Medium (MM)

To prepare 1 liter of medium, add the following to 950 ml of distilled water:

Salt mix Alacking $MgSO_4$@ (20x stock)	50	ml
$MgSO_4$ solution (0.4 M, 200x stock)	5	ml
Glucose	10	g
Trace element stock solution	1	ml

Mix in a 2-liter flask. The resulting pH will be ~ 6.6, and no adjustment will be needed.

For solid media, add agar (15 g/liter); weigh out into flasks as required. Mix, dispense, supplement as needed, and autoclave; swirl vigorously while hot to mix well.

[d] Alternatively, the phosphates can be kept separately and the other salts prepared as a mixture (G. May, personal communication).

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