Summary of Literature on Nutrient Media Used in Culturing Liverworts

Geldreich, Edwin E., Jr.
SUMMARY OF LITERATURE ON NUTRIENT MEDIA USED IN CULTURING LIVERWORTS

EDWIN E. GELDREICH, JR.,*  
Department of Botany, University of Cincinnati,  
Cincinnati 21, Ohio

During the course of recent investigations into the growth of liverworts, it was found that the information pertaining to the nutrient solutions, which had been used, was widely scattered and often difficult to find. In the following an attempt has been made to give a brief survey of this material in such a way that the composition of the various media may be compared and evaluated. In several instances when the quantities were not given in grams per liter, this data has been added in parentheses in a third column, for the purpose of conformity and comparison. The arrangement is chronological.

Marchal (1906) reported that he had cultured *Cephalozia byssacea* successfully on the following nutrient solution:

\[
\begin{align*}
\text{NH}_4\text{(NO}_3\text{)} & \quad 1.00 \text{ g.} \\
\text{K}_2\text{SO}_4 & \quad 0.50 \\
\text{CaSO}_4 & \quad 0.50 \\
\text{MgSO}_4 & \quad 0.50 \\
\text{K}_2\text{HPO}_4 & \quad 0.50 \\
\text{Fe(SO}_4\text{)}_3 & \quad 0.01 \\
\text{Distilled water} & \quad 1000.00 \text{ ml.} \\
\end{align*}
\]

Adjusted to pH 7.0 with 10 per cent KOH.

Dachnowski (1907) used Knop's solution modified in concentrations of 0.1 per cent to 0.4 per cent, with 0.3 per cent used most often, for culturing *Marchantia polymorpha* in the study of the development of rhizoids and the formation of gemmae. This was made up of the following:

\[
\begin{align*}
\text{MgSO}_4 & \quad 0.0075 \text{ g.} \\
\text{Ca(NO}_3\text{)}_2 & \quad 0.0300 \\
\text{K}_2\text{HPO}_4 & \quad 0.0075 \\
\text{KCl} & \quad 0.0036 \\
\text{FeCl}_3 & \quad \text{trace} \\
\text{Distilled water} & \quad 1000.00 \text{ ml.} \\
\end{align*}
\]

Osterhout (1907) used two nutrient solutions for the culturing of gemmae of *Lunularia* successfully for 200 days,—the duration of the experiment.

**Nutrient Solution A**

<table>
<thead>
<tr>
<th>cc. of 3/32 Molar</th>
<th>cc.</th>
<th>g.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>1000</td>
<td>(5.4803)</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>78</td>
<td>(0.6964)</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>38</td>
<td>(0.4288)</td>
</tr>
<tr>
<td>KCl</td>
<td>22</td>
<td>(0.1538)</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>10</td>
<td>(0.1040)</td>
</tr>
<tr>
<td>Distilled water</td>
<td></td>
<td>(1000 ml.)</td>
</tr>
</tbody>
</table>

On solution A, which was diluted artificial sea water,² there was a 1204 per cent increase in the length of the thallus. Another solution (solution B) which he also used gave almost equal results, a 980 per cent increase in the length of the *Lunularia* thallus.

**Nutrient Solution B**

<table>
<thead>
<tr>
<th>cc. of 3/32 Molar</th>
<th>cc.</th>
<th>g.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>1000</td>
<td>(5.4803)</td>
</tr>
<tr>
<td>KCl</td>
<td>22</td>
<td>(0.1538)</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>10</td>
<td>(0.1040)</td>
</tr>
<tr>
<td>Distilled water</td>
<td></td>
<td>(1000 ml.)</td>
</tr>
</tbody>
</table>

---

¹I wish to express my appreciation to Dr. Margaret Fulford for much helpful criticism in reading the manuscript.

²The artificial sea water was prepared from Van't Hoff's formula which has the same constituents but at ¾ M. strength.
Killian (1911) reported favorable results using a nutrient solution devised by Marchal for the study of cultures of hepatics. This included the following:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{NH}_4(\text{NO}_3)$</td>
<td>1.00 g.</td>
</tr>
<tr>
<td>$\text{K}_2(\text{SO}_4)$</td>
<td>0.50</td>
</tr>
<tr>
<td>$\text{Mg}(\text{SO}_4)$</td>
<td>0.50</td>
</tr>
<tr>
<td>$\text{Ca}(\text{SO}_4)$</td>
<td>0.50</td>
</tr>
<tr>
<td>$\text{Fe} (\text{SO}_4)_2$</td>
<td>0.01</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000.00 ml.</td>
</tr>
</tbody>
</table>

Buch (1920) reported good results with a nutrient solution which he had used in a morphological and physiological study of *Sphenolobus Michauxi*, *Pellia epiphylla*, *Blepharozia ciliaris*, and *Cephalosia bicuspidata*. It contained the following:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{K}_2\text{HPO}_4$</td>
<td>0.80 g.</td>
</tr>
<tr>
<td>$\text{MgSO}_4$</td>
<td>0.30</td>
</tr>
<tr>
<td>$\text{CaCl}_2$</td>
<td>0.30</td>
</tr>
<tr>
<td>$\text{FeCl}_3$</td>
<td>trace</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000.00 ml.</td>
</tr>
</tbody>
</table>

For his studies with the protonema of these species, Buch altered his nutrient solution and made a solid medium with the addition of agar as follows:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{KNO}_3$</td>
<td>0.12 per cent. (1.20 g.)</td>
</tr>
<tr>
<td>$\text{K}_2\text{HPO}_4$</td>
<td>0.08 (0.80 g.)</td>
</tr>
<tr>
<td>$\text{MgSO}_4$</td>
<td>0.03 (0.30 g.)</td>
</tr>
<tr>
<td>$\text{CaCl}_2$</td>
<td>0.03 (0.30 g.)</td>
</tr>
<tr>
<td>$\text{FeCl}_3$</td>
<td>trace</td>
</tr>
<tr>
<td>Agar</td>
<td>2.00 (20.0 g.)</td>
</tr>
<tr>
<td>Distilled water</td>
<td>(1000 ml.)</td>
</tr>
</tbody>
</table>

Lilienstern (1927) used both Uspenski's and Detmer's solutions plus two per cent agar for culturing *Marchantia polymorpha* in a morphological and physiological study. The composition of these two nutrient solutions is given below:

### Uspenski Nutrient Solution

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{KNO}_3$</td>
<td>0.02500 g.</td>
</tr>
<tr>
<td>$\text{MgSO}_4$</td>
<td>0.02500 g.</td>
</tr>
<tr>
<td>$\text{Ca} (\text{NO}_3)_2$</td>
<td>0.10000 g.</td>
</tr>
<tr>
<td>$\text{KH}_2\text{PO}_4$</td>
<td>0.02500 g.</td>
</tr>
<tr>
<td>$\text{K}_2\text{CO}_3$</td>
<td>0.03450 g.</td>
</tr>
<tr>
<td>$\text{Fe} (\text{SO}_4)_3$</td>
<td>0.00125 g.</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000.00 ml.</td>
</tr>
<tr>
<td>pH of nutrient solution</td>
<td>7.6.</td>
</tr>
</tbody>
</table>

### Detmer Nutrient Solution

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{Ca} (\text{NO}_3)_2$</td>
<td>1.00 g.</td>
</tr>
<tr>
<td>$\text{MgCl}_2$</td>
<td>0.25</td>
</tr>
<tr>
<td>$\text{MgSO}_4$</td>
<td>0.25</td>
</tr>
<tr>
<td>$\text{KH}_2\text{PO}_4$</td>
<td>0.25</td>
</tr>
<tr>
<td>$\text{FeCl}_3$</td>
<td>trace</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000.00 ml.</td>
</tr>
<tr>
<td>The pH of the solution</td>
<td>6.8.</td>
</tr>
</tbody>
</table>

Ehring (1934) used the following four solutions on *Marchantia polymorpha*, *Lunularia cruciata*, and *Riccia fluitans* with success.

### "a" Nutrient Solution

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{NaNO}_3$</td>
<td>0.0200 per cent. (0.200 g.)</td>
</tr>
<tr>
<td>$\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$</td>
<td>0.0100 (0.100 g.)</td>
</tr>
<tr>
<td>$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$</td>
<td>0.0100 (0.100 g.)</td>
</tr>
<tr>
<td>$\text{KH}_2\text{PO}_4$</td>
<td>0.0100 (0.100 g.)</td>
</tr>
<tr>
<td>$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$</td>
<td>0.0005 (0.005 g.)</td>
</tr>
<tr>
<td>Distilled water</td>
<td>(1000 ml.)</td>
</tr>
<tr>
<td>(Approximate salt concentration 0.05 per cent.)</td>
<td></td>
</tr>
</tbody>
</table>
### "β" Nutrient Solution

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNO₃</td>
<td>0.010 per cent</td>
<td>0.10 g</td>
</tr>
<tr>
<td>CaSO₄ · 2H₂O</td>
<td>0.020</td>
<td>0.20 g</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>0.020</td>
<td>0.20 g</td>
</tr>
<tr>
<td>MgSO₄ · 7H₂O</td>
<td>0.020</td>
<td>0.20 g</td>
</tr>
<tr>
<td>FeO₂</td>
<td>0.003</td>
<td>0.03 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>(1000 ml)</td>
<td></td>
</tr>
</tbody>
</table>

(Approximate salt concentration 0.07 per cent.)

### "γ" Nutrient Solution

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNO₃</td>
<td>0.1000 per cent</td>
<td>1.000 g</td>
</tr>
<tr>
<td>Ca₃(PO₄)₂</td>
<td>0.0500</td>
<td>0.500 g</td>
</tr>
<tr>
<td>MgSO₄ · 7H₂O</td>
<td>0.0500</td>
<td>0.500 g</td>
</tr>
<tr>
<td>FeSO₄ · 7H₂O</td>
<td>0.0005</td>
<td>0.005 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>(1000 ml)</td>
<td></td>
</tr>
</tbody>
</table>

(Approximate salt concentration 0.2 per cent.)

### "δ" Nutrient Solution

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄NO₃</td>
<td>0.020 per cent</td>
<td>0.20 g</td>
</tr>
<tr>
<td>Ca₃(PO₄)₂</td>
<td>0.020</td>
<td>0.20 g</td>
</tr>
<tr>
<td>KCl</td>
<td>0.020</td>
<td>0.20 g</td>
</tr>
<tr>
<td>MgSO₄ · 7H₂O</td>
<td>0.020</td>
<td>0.20 g</td>
</tr>
<tr>
<td>FeSO₄ · 7H₂O</td>
<td>0.002</td>
<td>0.02 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>(1000 ml)</td>
<td></td>
</tr>
</tbody>
</table>

(Approximate salt concentration 0.08 per cent.)

Müller (1939) reported a nutrient solution used by Lorbeer which is a nutrient agar modification of the one used by Benecke.

MgSO₄ · 7H₂O | 0.200 g |
CaCl₂         | 0.100 |
KH₂PO₄       | 0.100 |
MgSO₄        | 0.100 |
FeCl₃ · 3H₂O | 0.005 |
Agar          | 15.00 |
Distilled water | 1000.00 ml |

(The pH of the solution was between 5 and 6.)

Also in the same year, Griggs (1939) reported a nitrogen free solution on which he had cultured *Cephaloziella byssacea* successfully for three years. The solution was a modification of one of the three salt nutrient solutions devised by Shive, but with only two-fifths the concentration.

KH₂PO₄ | 1.225 g |
MgSO₄ · 7H₂O | 1.848 |
CaSO₄ (anhydrous) | 0.340 |
iron as:
- ferric phosphate, or
- ferric chloride, or
- ferric citrate, | trace |
Distilled water | 1000.00 ml |

(Voth and Hamner (1940) used the following nutrient solution successfully in a physiological study of *Marchantia polymorpha*.)

MgSO₄ | 0.1204 g |
MgHPO₄ · 3H₂O | 0.1744 |
Mg(NO₃)₂ · 6H₂O | 0.2564 |
CaSO₄ | 0.1722 |
Ca₃(PO₄)₂ | 0.1261 |
Ca(NO₃)₂ | 0.2302 |
K₂SO₄ | 0.1742 |
KH₂PO₄ | 0.2723 |
KNO₃ | 0.2022 |
Trace elements | 1.00 ml |

(Osmotic concentration approximately 0.285 atmos.)
Since then, we have successfully cultured plants of *Leucoblejeunea clypeata*, for five months, on the nutrient solution described by Voth and Hamner above. Voth (1941) later suggested the following nutrient solution as the one best for culturing *Marchantia polymorpha*.

\[
\begin{align*}
&\text{cc. of 0.5 Molar} \\
&\text{KNO}_3 & 1.6 \quad (0.0808 \text{ g.}) \\
&\text{Ca(NO}_3)_2 & 1.4 \quad (0.1148 \text{ g.}) \\
&\text{Mg (NO}_3)_2 & 1.2 \quad (0.0890 \text{ g.}) \\
&\text{KH}_2\text{PO}_4 & 0.8 \quad (0.0544 \text{ g.}) \\
&\text{MgSO}_4 & 1.6 \quad (0.0992 \text{ g.}) \\
&\text{Distilled water} & (1000 \text{ ml.}) \\
\end{align*}
\]

Voth (1941) later suggested the following nutrient solution as the one best for culturing *Marchantia polymorpha*.

\[
\begin{align*}
&\text{NH}_4\text{NO}_3 & 0.200 \text{ g.} \\
&\text{CaCl}_2 & 0.100 \\
&\text{KH}_2\text{PO}_4 & 0.100 \\
&\text{MgSO}_4 \cdot 7\text{H}_2\text{O} & 0.100 \\
&\text{FeCl}_3 \cdot 6\text{H}_2\text{O} & 0.005 \\
&\text{Agar} & 8.00 \\
&\text{Distilled water} & 1000.00 \text{ ml.} \\
\end{align*}
\]

Prat also stated that *Riella* was cultured in erlenmeyer flasks on sand to which the above nutrient solution was added, minus the agar.

**LITERATURE CITED**


