A green micro-algal growth media modified for use as a stringent minimal media for bacteria.

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ABSTRACT

Introduction:

Chlamydomonas reinhardtii, a green micro-alga can be grown at the lab heterotrophically or photo-heterotrophically at room temperature using the Tris-Phosphate-Acetate (TAP) medium which contains 0.1 % acetate (acetic acid) as the sole carbon source. Standard TAP medium recipe can be found at the website of Chlamydomonas Resource Center: https://www.chlamycollection.org/methods/media-recipes/tap-and-tris-minimal. Hutner’s trace element solution is an ingredient in the TAP medium. Hutner’s trace element recipe can be found at https://www.chlamycollection.org/methods/media-recipes/hutners-trace-elements/. M9 medium is the standard minimal medium for growing bacteria (http://www.thelabrat.com/protocols/m9minimal.shtml). When grown in TAP medium, Chlamydomonas can utilize the exogenous acetate in the medium to make net biosynthesis of sugar using the Glyoxylate cycle and can grow either heterotrophically or photo-heterotrophically without being dependent on photosynthesis for glucose biosynthesis. Higher green plants have the glyoxylate cycle which they use to make sugar from internal pool of acetyl CoA but cannot take up exogenous acetate like Chlamydomonas.

In our lab we use a slightly modified TAP medium recipe which has a final concentration of phosphate, nitrogen, magnesium and calcium approximately 10-fold, 2-fold, 2-fold and 2-fold higher than that in the TAP recipe described on the Chlamydomonas Resource Center website, respectively. Final concentrations of acetate and Hutner’s trace elements are same in both TAP recipes. Attached Table 1 compares the chemical ingredients in our lab’s TAP recipe (full recipe can be found on protol.io) with that present in the standard M9 medium described at http://www.thelabrat.com/protocols/m9minimal.shtml. M9 medium has a final concentration of phosphate, nitrogen, magnesium and carbon, approximately 70-fold, 2.5-fold, 4-fold and 5-fold higher than that present in our lab’s TAP medium, respectively. Additionally, M9 contains 0.05% salt (8.56 mM) (Table 1). TAP has additional trace elements like iron, zinc, copper, manganese, cobalt, boron and molybdenum which are components in the Hutner’s trace element solution (Table1). In summary, TAP medium is chemical composition wise, a more stringent minimal medium than the M9 medium used by microbiologists.

Applications:

There are many bacteria that can also utilize acetate as a carbon source. Some of these bacteria also have glyoxylate cycle like Chlamydomonas and green plants. Hence, TAP medium can be used to isolate acetate-requiring bacteria. If acetate is removed from the TAP medium, it becomes the TP medium which lacks a carbon source and will not allow Chlamydomonas or other acetate-requiring bacteria to grow. The 0.1 % acetate in the TAP medium can be substituted with alternative carbon sources (e.g. glucose, sucrose, lactose, hydrocarbons, aromatic compounds and polyhydroxyalkanoates etc.) to test physiological abilities of candidate bacteria to use the tested chemicals as the sole alternative carbon and energy source.

EXTERNAL LINK

https://f1000research.com/articles/9-656/v1


ATTACHMENTS
Table 1.pdf

DOI
dx.doi.org/10.17504/protocols.io.bgzuix6w

EXTERNAL LINK
https://f1000research.com/articles/9-656/v1

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KEYWORDS
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37652
GUIDELINES

Do not use NaOH for pHing Hutner’s trace element. Use KOH pellets and solution for pHing Hutner’s trace element.

MATERIALS TEXT

MATERIALS

- Cobaltous chloride hexahydrate Contributed by users
- Zinc sulfate heptahydrate Sigma
- Aldrich Catalog #204986
- Boric acid Fisher
- Scientific Catalog #BP1681
- Manganese chloride Fisher
- Scientific Catalog #7773-01-5
- Ammonium molybdate (VI) tetrahydrate Fisher
- Scientific Catalog # 12054-85-2
- Calcium chloride, dihydrate Bio Basic
- Inc. Catalog #CD0050.SIZE.500g
- Copper (II) sulfate pentahydrate Bio Basic
- Inc. Catalog #CDB0063.SIZE.500g
- Iron (II) sulfate, heptahydrate Bio Basic
- Inc. Catalog #FB0461.SIZE.500g
- Potassium hydroxide Bio Basic
- Inc. Catalog #PB0441.SIZE.500g
- Magnesium Sulfate Heptahydrate, ACS Grade Gold
- Biotechnology Catalog #M-020
- Acetic acid Glacial Fisher
- Scientific Catalog #A38-212
- Tris Base Fisher
- Scientific Catalog #604204
- Potassium phosphate Dibasic Sigma Aldrich
- Scientific Catalog #5295681KG
- Potassium Phosphate, Monobasic, Molecular Biology Grade, Calbiochem™, 1kg Thermo
- Fisher Catalog #A661-500

BEFORE STARTING

You can purchase all chemicals needed to make the TAP medium from any vendor that sells them and do not have to use the vendors that I have selected under "Materials”.

Preparation of TAP stock nutrients

1. Make TAP salts (TAP stock nutrients/FBS solution [Filner’s Beijerinck solution X40]) by dissolving Ammonium chloride (16g), Magnesium sulfate (4g) and Calcium chloride (2.65g). This will give you a final concentration of: Ammonium chloride (7.48 mM), Magnesium sulfate (16.2 mM) and Calcium chloride (18.10 mM).

Citation: Mautusi Mitra (06/12/2020). A green micro-algal growth media modified for use as a stringent minimal media for bacteria. https://dx.doi.org/10.17504/protocols.io.bgzujx6w

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Preparation of Phosphate solution

2. Make Phosphate solution (1M Potassium phosphate solution, pH 7) by dissolving 28.8 g of \( \text{K}_2\text{HPO}_4 \) and 14.4 g of \( \text{KH}_2\text{PO}_4 \). **Note:** You don’t have to adjust pH for this solution as it automatically gives a pH of 7-7.2 when mixed in the above stated ratio. Phosphate solution has the final concentration of \( \text{K}_2\text{HPO}_4 \) (1.65M) and \( \text{KH}_2\text{PO}_4 \) (1.058M).

Preparation of Hutner’s trace element

3. Preparation of Hutner’s trace element (X 1000) (Note: If you do not want to make it, you can purchase this solution from Chlamydomonas Resource center at [https://www.chlamycollection.org/methods/media-recipes/hutnerstrace-elements/](https://www.chlamycollection.org/methods/media-recipes/hutnerstrace-elements/). In order to make this solution, dissolve the following salts in order in 800 mL of E-pure water—dissolve each fully before adding the next.

<table>
<thead>
<tr>
<th>Salt</th>
<th>Molecular Weight</th>
<th>Final concentration in 1L</th>
<th>Amount to be added in 1L</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeSO4.7H2O</td>
<td>278.01</td>
<td>18 mM</td>
<td>4.99 g</td>
</tr>
<tr>
<td>ZnSO4.7 H2O</td>
<td>287.56</td>
<td>76.5mM</td>
<td>22 g</td>
</tr>
<tr>
<td>H3BO3</td>
<td>61.83</td>
<td>184 mM</td>
<td>11.4 g</td>
</tr>
<tr>
<td>MnCl2.4H2O</td>
<td>197.91</td>
<td>25.6 mM</td>
<td>5.06 g</td>
</tr>
<tr>
<td>CuSO4.5H2O</td>
<td>249.68</td>
<td>6.3mM</td>
<td>1.57 g</td>
</tr>
<tr>
<td>(NH4)6Mo7O24.4 H2O</td>
<td>1235.86</td>
<td>0.89mM</td>
<td>1.10 g</td>
</tr>
<tr>
<td>CoCl2.6H2O</td>
<td>237.93</td>
<td>6.8mM</td>
<td>1.61 g</td>
</tr>
</tbody>
</table>

Table of chemical ingredients needed to make Hutner’s trace element.

4. Bring the salt mixture to a slow boil. Add 50 grams of disodium salt of EDTA to the boiling mixture, acid form (Na\(_2\)EDTA.2H\(_2\)O; molecular weight is 372.24; final concentration in the 1L solution is 134 mM).

5. Add KOH pellets (**and not NaOH**) to the boiling mixture to adjust the pH to 6.5. Make up the volume to 1L with pure water after adjusting the pH to 6.5. The solution should be clear green at this point.

6. Pour the green solution in a 1 L bottle. Close the cap not too tightly. Shake it occasionally every week. The color will slowly change to dark magenta/purplish color over time. If you see any brown precipitate, filter the solution through two layers of Whatman#1 filter paper, repeating the filtration if necessary until the solution is clear.

Preparation of TAP medium

7. To make the final TAP medium, mix the following in 950 L of water:
   2.42 g Tris base/Trizma, 25 ml solution #1 (salts), 0.375 ml solution #2 (phosphate), 1.0 ml solution #3 (trace elements) and 1.0 ml glacial acetic acid (0.1% in the final 1 L of TAP medium).

8. Check the pH of the solution before making up the volume to 1L. pH of TAP medium should be approximately between 7-7.2 once you make it but if it is not, adjust pH with acid/base. The final concentration of all chemical ingredients that you have added to make TAP medium is given in the attached Table 1 pdf.

Preparation of TAP-agar plates

9. For preparing solid TAP-agar media plates to maintain *Chlamydomonas* in lab, add 15 grams of agar per liter of the media after checking the pH, shake it and then autoclave. **Note:** Make sure to have a magnetic stir bar inside the agar media bottle before you autoclave the bottle. Stirring using a magnetic stirrer is required during the cooling of the hot media after autoclaving to mix agar uniformly in the media solution before pouring media plates.

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Preparation of TP medium

TP medium is prepared exactly the same way as the TAP medium, except glacial acetic acid is not added. Hence TP medium has no carbon source. Nothing will grow on it unless you add a carbon source to the TP medium. You will pH the TP medium like you would for making TAP medium. You can add your preferred alternative carbon source (e.g. glucose, sucrose, lactose, hydrocarbons, aromatic compounds and polyhydroxyalkanoates etc.) for testing biochemical abilities of bacteria to use exogenous carbon sources for energy production and growth.