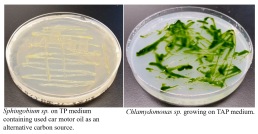
Jun 12, 2020

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



F1000Research

DOI
dx.doi.org/10.17504/protocols.io.bgzujx6w

Mautusi Mitra[1]

[1]University of West Georgia

Mautusi Mitra

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.bgzujx6w

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

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

Manuscript citation:
1. Mitra M, Nguyen KMAK, Box TW *et al.* Isolation and characterization of a novel *Sphingobium yanoikuyae* strain variant that uses biohazardous saturated hydrocarbons and aromatic compounds as sole carbon sources [version 1; peer review: 2 approved]. *F1000Research* 2020, **9**:767 (https://doi.org/10.12688/f1000research.25284.1)

2. Mitra M, Nguyen KMAK, Box TW *et al.* Isolation and characterization of a novel bacterial strain from a Tris-Acetate-Phosphate agar medium plate of the green micro-alga *Chlamydomonas reinhardtii* that can utilize common environmental pollutants as a carbon source [version 1; peer review: 3 approved]. *F1000Research* 2020, **9**:656 (https://doi.org/10.12688/f1000research.24680.1)

Protocol status: Working
We use this protocol in our group and it is working.

Created: May 31, 2020

Last Modified: June 12, 2020

Protocol Integer ID: 37652

Keywords: TAP medium, M9 medium, Chlamydomonas reinhardtii, alternative carbon source, bacteria grown in TAP medium, TP medium, acetate-consuming bacteria, alternative carbon souces, bacterial minimal medium

Abstract

Introduction:
Chlamydomonas reinhardtii, a green micro-alga can be grown at the lab heterotrophically or photo-heterotrophically at room temperature using the **T**ris-**P**hosphate-**A**cetate (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.

Attachments

PDF

Table 1.pdf

40KB

Guidelines

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

Materials

MATERIALS

- Cobaltous chloride hexahydrate
- 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**
- Potassium Phosphate, Monobasic, Molecular Biology Grade, Calbiochem™, , 1kg **Thermo Fisher** **Catalog #**5295681KG
- Ammonium Chloride **Fisher Scientific** **Catalog #**A661-500

Before start

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).

Preparation of Phosphate solution

2 Make Phosphate solution (1M Potassium phosphate solution, pH7) by dissolving 28.8 g of K_2HPO_4 and 14.4g of KH_2PO_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 concnetration of K_2HPO_4 (1.65M) and KH_2PO_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 at **https://www.chlamycollection.org/methods/media-recipes/hutners-trace-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.

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

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.$2H_2O$; 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 sof 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.

Preparation of TP medium

10 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 souce. 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 abilites of bacteria to use exogenous carbon sources for energy production and growth.