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Culturing *Symbiodinium*

In 1 collection

Tingting Xiang¹, Arthur Grossman²

¹University of California, Riverside; ²Carnegie Institution for Science

Aiptasia Symbiodiniaceae Model System

Tingting Xiang

University of California, Riverside

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ABSTRACT

we generated five clonal, axenic strains of *Symbiodinium*. These strains were assigned to clades A (two strains), B, E, and F based on their chloroplast 23S rDNA sequences. Growth studies in liquid cultures showed that the clade B strain and one of the clade A strains were able to grow photoautotrophically (in light with no fixed carbon), mixotrophically (in light with fixed carbon), or heterotrophically (in dark with fixed carbon). Here this protocol describes the steps of culturing SSB01 in different types of medium as an example.

MATERIALS

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CORAL PRO SALT Contributed by users Catalog #R_99344

DAIGOS IMK MEDIUM FOR MARINE MICROALGAE Wako Catalog #398-01333

Glucose Sigma Aldrich Catalog #G8270

Marine Broth 2216 BD Biosciences Catalog #279110

Noble agar Sigma Aldrich Catalog #A5431

Enzymatic Digest Casein Hydrolysate Contributed by users Catalog #AAJ12855Q1

Prepare liquid media

- 1 Artificial seawater (ASW) is the base for most of the media, except Marine Broth.

Making ASW

- 2 Dissolve 37.1 g Coral Pro salt into 1L pure water; check the salinity of the dissolved seawater and make sure it is between 32-34.

Note

This step of dissolving sea salts may take a while, stir overnight is recommended.

Making 10x Daigo stock solution for the media

- 3 Dissolve 2.56g Daigo's IMK Medium powder into 1L ASW;

Note

Daigo's IMK Medium is the base of IMK medium.

Making 10x Daigo's IMK Medium stock solution

- 4 Filter sterile the 10X Daigo's IMK Medium solution through a 0.22 μ m filter;

Note

Here is the recipe of Daigo' IMK Medium, which contains nutrients such as vitamins. Therefore, filter sterilization is highly recommended instead of autoclave sterilization.

〔Ⅳ〕細胞培養用培地

微細藻類培養用

海洋微細藻類培養用

ダイゴIMK培地

Daigo's IMK Medium for Marine Microalgae

【和光コード・包装】398-01333（100L用×10）

概要

本培地は、多大な微細藻類をできるだけ多く確実に効率よく培養可能とする培地を簡便に調製できるよう、「株式会社海洋バイオテクノロジー研究所」が開発した海産微細藻類用の培地です。

海水に溶かすだけで、培地の調製が可能のように、全ての要素物質が混合されています。

環境中の微細藻類の分離から大量培養^(B)まで広範に利用可能で、海産微細藻類培養、生理学的研究、水産養殖における稚苗生産用の飼料藻類の培養等に便利な培地として設計されています。

(注)培養用の高密度培養には、別途塩酸ナトリウム (0.2~1M) を添加してください。

組成・成分 (mg/L)

NaNO ₃	200	CoSO ₄ · 7H ₂ O	0.014
Na ₂ HPO ₄	1.4	Na ₂ MoO ₄ · 2H ₂ O	0.0073
K ₂ HPO ₄	5	CuSO ₄ · 5H ₂ O	0.0025
NH ₄ Cl	2.68	H ₂ SeO ₃	0.0017
Fe-EDTA	5.2	Thiamin-HCl	0.2
Mn-EDTA	0.332	Biotin	0.0015
Na ₂ -EDTA	37.2	Vitamin B ₁₂	0.0015
ZnSO ₄ · 7H ₂ O	0.023	MnCl ₂ · 4H ₂ O	0.18

使用法

海水100L (1000L) に本品25.2g (252g) を攪拌しながら加えて溶解する。

※ pH調整を必要とする場合は、塩酸又は水酸化ナトリウムを用いる。pHがアルカリ側に傾くと白色沈殿を生ずることがある。

※ ダイゴIMK培地を用いて大量培養や高密度培養を行う場合は、1Lに対する粉末添加量を増やすことが可能である。

※ IMK培地を人工海水SPに溶解するとき、必ず人工海水SPを溶解した後にIMK培地を溶解してください。

1) 滅菌について

- 滅菌をしていない培地は、培地調製中に海水または空中の微細藻類が混入し、希培養においても微細藻類が発生することがある。
- ダイゴIMK培地には、ビタミン類が配合されているので、培地を滅菌する際には、培養対象のビタミン要求性を考慮して、メンブランフィルターを用いてろ過滅菌する。滅菌後は4℃で保管し、できるだけ早く使用すること。

2) カンテン培地の調製法

ダイゴ人工海水SP (海水) 800mLにダイゴIMK培地202mgを加え溶解し、滅菌後培地を60℃程度に保つ。別に、蒸留水200mLにカンテン15gを加え121℃で15分間高圧蒸気滅菌し、攪拌の後、室温で放置して60℃程度になったら、先に滅菌した培地とよく混合して、シャーレに分注する。固化後、希培養所に保管する。

注意事項

※本品は「医薬用外毒物」です。

本品は吸湿性が強いので開封後は密封して保管すること。室温保存。

Making liquid IMK medium

- 5 Transfer 90 mL ASW into a 250 mL PYREX Glass Erlenmeyer Flask, and cover with aluminum foil;
- 6 Autoclave at 121 °C for 30 minutes;
- 7 Let it cool to room temperature.
- 8 Bring the flask from step 7 and 10X Daigo's IMK Medium stock solution from step 4 to a sterile hood;
- 9 Add 10 mL Daigo's IMK Medium stock solution to the flask; and that makes 100 mL IMK.

Making liquid IMK medium supplemented with casein hydrop.

- 10 Dissolve 0.4 g casein hydrolysate into 90 mL ASW, and transfer to a clean 250 mL PYREX Glass Erlenmeyer Flask, and cover with aluminum foil;

Note

Please note to use enzymatic digest casein hydrolysate, the hydrochloric acid hydrolysate of casein may not dissolve well in ASW.

- 11 Autoclave at 121 °C for 30 minutes;
- 12 Let it cool to room temperature.

- 13 Bring the flask from step 12 and 10X Daigo's IMK Medium stock solution from step 4 to a sterile hood;
- 14 Add 10 mL Daigo's IMK Medium stock solution to the flask; and that makes 100 mL IMK+cas medium.

Making liquid IMK medium supplemented with glucose (IMK..

- 15 Dissolve 0.5 g glucose into 90 mL ASW, and cover with aluminum foil;
- 16 Autoclave at 121 °C for 30 minutes;
- 17 Let it cool to room temperature.
- 18 Bring the flask from step 17 and 10X Daigo's IMK Medium stock solution from step 4 to a sterile hood;
- 19 Add 10 mL Daigo stock solution to the flask; and that makes 100 mL IMK+Glc medium.

Making liquid Marine Broth (MB) medium

- 20 Dissolve 37.4 g MB powder into 1L pure water;
- 21 Transfer the liquid to a new 1L glass container;
- 22 Autoclave at 121 °C for 30 minutes; and let it cool down to room temperature.

Note

There might be some precipitation after autoclave, filter through a 0.45µm filter is recommended to get rid of the precipitation.

Inoculate the Symbiodinium culture

- 23 Pick single colonies from agar plates which contain the Symbiodinium colonies;

Note

Image showing Symbiodinium colonies on a solid plate.

- 24 Grow the cultures at 27°C under 10 µmol photons m⁻² s⁻¹ white light.

Note