Culturing Symbiodinium

Tingting Xiang,Arthur Grossman

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.

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Materials

- CORAL PRO SALT R_99344 by Contributed by users
- DAIGOS IMK MEDIUM FOR MARINE MICROALGAE 398-01333 by Wako
- Glucose G8270 by Sigma Aldrich
- Marine Broth 2216 279110 by BD Biosciences
- Noble agar A5431 by Sigma Aldrich
- Enzymatic Digest Casein Hydrolysate AAJ12855Q1 by Contributed by users

Protocol

Prepare liquid media

Step 1.

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

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Making ASW

Step 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.

NOTES

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

Making 10x Daigo stock solution for the media

Step 3.

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

NOTES

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

Making 10x Daigo's IMK Medium stock solution

Step 4.

Filter sterile the 10X Daigo's IMK Medium solution through a 0.22 µm filter;

NOTES

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

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微細藻類培養用

海産微細藻類培養用

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）
392-01331（1000Ｌ用）

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、培地の調製が可能なように、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(注)まで広範に利用可能で、海産微細藻類培養、生理学的研究、水産業における種苗生産用の餌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)珪藻類の高密度培養には、別途珪酸ナトリウム（0.2～1mM）を添加してください。

組成・成分（mg/L）

$NaNO_3$	200	$CoSO_4 \cdot 7H_2O$	0.014
Na_2HPO_4	1.4	$Na_2MoO_4 \cdot 2H_2O$	0.0073
K_2HPO_4	5	$CuSO_4 \cdot 5H_2O$	0.0025
NH_4Cl	2.68	H_2SeO_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
$ZnSO_4 \cdot 7H_2O$	0.023	$MnCl_2 \cdot 4H_2O$	0.18

使用法

海水100L（1000L）に本品25.2g（252g）を撹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、冷暗所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、ビタミン類が調合されているので、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252mgを加え溶解し、滅菌後培地を50℃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、撹拌の後、恒温で放置して50℃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、冷暗所に保管する。

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。

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Making liquid IMK medium

Step 5.

Transfer 90 mL ASW into a 250 mL PYREX Glass Erlenmeyer Flask, and cover with aluminum foil;

Making liquid IMK medium

Step 6.

Autoclave at 121 ºC for 30 minutes;

Making liquid IMK medium

Step 7.

Let it cool to room temperature.

Making liquid IMK medium

Step 8.

Bring the flask from step 7 and 10X Daigo's IMK Medium stock solution from step 4 to a sterile hood;

Making liquid IMK medium

Step 9.

Add 10 mL Daigo's IMK Medium stock solution to the flask; and that makes 100 mL IMK.

Making liquid IMK medium supplimented with casein hydroplysate (IMK+cas)

Step 10.

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

NOTES

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

Making liquid IMK medium supplimented with casein hydroplysate (IMK+cas)

Step 11.

Autoclave at 121 ºC for 30 minutes;

Making liquid IMK medium supplimented with casein hydroplysate (IMK+cas)

Step 12.

Let it cool to room temperature.

Making liquid IMK medium supplimented with casein hydroplysate (IMK+cas)

Step 13.

Bring the flask from step 12 and 10X Daigo's IMK Medium stock solution from step 4 to a sterile hood;

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Making liquid IMK medium supplimented with casein hydroplysate (IMK+cas)

Step 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+Glc)

Step 15.

Dissolve 0.5 g glucose into 90 mL ASW, and cover with aluminum foil;

Making liquid IMK medium supplemented with glucose (IMK+Glc)

Step 16.

Autoclave at 121 ºC for 30 minutes;

Making liquid IMK medium supplemented with glucose (IMK+Glc)

Step 17.

Let it cool to room temperature.

Making liquid IMK medium supplemented with glucose (IMK+Glc)

Step 18.

Bring the flask from step 17 and 10X Daigo's IMK Medium stock solution from step 4 to a sterile hood;

Making liquid IMK medium supplemented with glucose (IMK+Glc)

Step 19.

Add 10 mL Daigo stock solution to the flask; and that makes 100 mL IMK+Glc medium.

Making liquid Marine Broth (MB) medium

Step 20.

Dissolve 37.4 g MB powder into 1L pure water;

Making liquid Marine Broth (MB) medium

Step 21.

Transfer the liquid to a new 1L glass container;

Making liquid Marine Broth (MB) medium

Step 22.

Autoclave at 121 ºC for 30 minutes; and let it cool down to room temperature.

NOTES

There might be some precipitation after autoclave, filter through a 0. 45µm filter is recommended to get rid of the precipitation.

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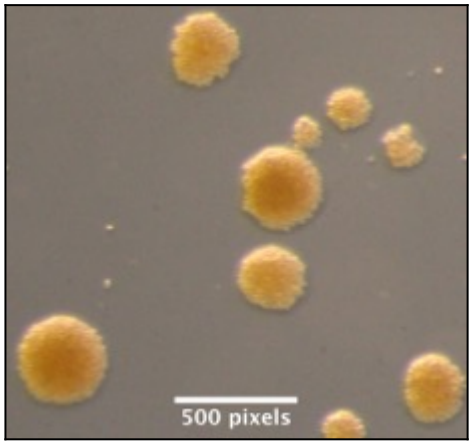
Inoculate the Symbiodinium culture

Step 23.

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

NOTES

Image showing Symbiodinium colonies on a solid plate.



Inoculate the Symbiodinium culture

Step 24.

Grow the cultures at 27ºC under 10 µmol photons m^{-2} s^{-1} white light.

NOTES

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