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Deep Ocean Water Applications Society

海洋深層水研究

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第 17 回海洋深層水利用学会全国大会

17th Annual Meeting of Deep Ocean Water Applications Society

海洋深層水 2013　台湾大会

講演要旨集

Abstracts

主催：海洋深層水利用学会

Organizer: Deep Ocean Water Applications Society

後援：財団法人石材・資源産業研究発展センター

Sponsor: Stone & Resource Industry R&D Center

第１７回　海洋深層水利用学会　全国大会海洋深層水　２０１３台湾大会　プログラム
Program of the 17th Annual Meeting of Deep Ocean Water Applications Society

１１月２日（土）
November 2nd (Sat.)

Ⅰ　オープニング（Opening Ceremony）

9：00　－　9：05　開会挨拶
海洋深層水利用学会会長
高橋　正征　（東京大学名誉教授・高知大学名誉教授）
President of Deep Ocean Water Applications Society
Masayuki Takahashi
(Emeritus professor of the University of Tokyo and Kochi University)

9：05　－　9：10　台湾経済部工業局局長
沈　栄津
Director General of Industrial Development Bureau, MOEA, Taiwan
Jong-Chin Shen

9：10　－　9：15　財団法人　石材・資源産業研究発展センター董事長
徐　福栄
President of Stone and Resource Industry R&D Center
Fu-Jong Hsu

9：15　－　9：35　来賓の祝辞

9：15　－　9：20　花蓮県県長
傅　崐萁
Governor of Hualien County
Kun-Chi Fu

9：20　－　9：25　立法委員
王　廷升
Member of The Legislative Yuan, Taiwan
Ting-Son Wang

9：25　－　9：30　台湾深層海水発展協会理事長
林　慶明
President of Taiwan Deep Sea Water Development Association
Ching-Ming Lin

9：30　－　9：35　国立東華大学学長
呉　茂昆
President of National Dong Hwa University
Maw-Kuen Wu

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１１月３日（日）

November 3rd (Sun)

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（国立台湾師範大学　生命科学学科　特任教授）

Chairman: Chiang-Ting Chien

(Department of Life Science, National Taiwan Normal University, Distinguished Professor)

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１１月４日（月）

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■　**見学会(Excursion tour)**

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特別シンポジウム

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司会　　大内　一之

（東京大学大学院新領域創成科学研究科・特任研究員）

Chairman: Kazuyuki Ouchi

(Graduate School of Frontier Sciences, The University of Tokyo, Project Researcher)

S1. 海洋立國：談台灣海洋產業發展與遠景

黃 煌雄(中華民國監察委員)

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—出版「海洋與台灣」四本叢書
（二）第二次「海洋與台灣」學術研討會（2009 年 4 月
—發表「台北海洋宣言」
—出版「海洋台灣」三本叢書
（三）「海洋與台灣相關課題總體檢」(2002 年 9 月—2004 年 4 月）
—歷時 18 個月，全文超過 20 萬字
—提出 6 項糾正，33 項調查意見
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（四）「深層海水資源利用及產業發展實施計畫」(2006 年 1 月 經濟部）
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1994 年 聯合國海洋公約正式生效
1997 年 聯合國訂為國際珊瑚礁年
1998 年 聯合國訂為國際海洋年
2001 年 巴黎召開全球海洋會議
2002 年 南非召開永續發展高峯會
2003 年 馬來西亞召開東亞海洋會議
2008 年 越南召開：「第四屆全球海洋・海岸與島嶼會議」
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2004 年 行政院頒布「國家海洋政策綱領」
2004 年 監察院提出「海洋與台灣相關課題總體檢」調查報告
2006 年 行政院出版第二本「海洋政策白皮書」
2007 年 教育部出版第一本「海洋教育白皮書」
2008 年 馬英九先生提出「藍色革命、海洋興國」的競選主張
2010 年 行政院已通過設置「海洋委員會」

S1. Ocean Country: The Development and Vision of Taiwanese Marine Industries

°Huang-Hsiung Huang (Member of Control Yuan, Taiwan)

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— Issued the Kaohsiung Marine Declaration

— Published four *Ocean and Taiwan* book series

(2) The second Ocean and Taiwan Academic Symposium (April 2009)

— Issued the Taipei Marine Declaration

— Published three Ocean Taiwan book series

(3) *Comprehensive Examination of Topics Related to Oceans and Taiwan* (September 2002–April 2004)

— The study lasted for 18 months and published more than 200,000 words

— Proposed six revisions and 33 survey comments

Including: Although domestic strategic industries have been promoted for numerous years, current fields in strategic marine industries are lacking. This has caused poor performance in the development of marine resources and severely affected the promotion of strategic marine industries, indicating development neglect and failure.

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S2. 海洋深層水資源が支える21世紀の社会

○高橋　正征
（海洋深層水利用学会会長、東京大学・高知大学名誉教授）

1．はじめに

人類の歴史を見ると、個人の物質的な豊かさの飛躍が顕著だ。最も根本的な理由は、科学・技術の進歩によって地下資源を中心とした資源利用の進んだことが大きい。しかし、地下資源は容易に資源利用ができた反面、（1）資源の減少・枯渇と、（2）地下資源の利用による廃棄物で生物圏の汚染（環境問題）という深刻な問題を生んだ。今後は、非再生の地下資源から、身近にある再生資源への切り替えが喫緊の課題である。再生資源の多くは、資源密度が低く、利用効率の向上技術の開発と、利用方法の工夫が課題である。

海洋深層水（以下、深層水）は、水深約200m以深で光が十分に射しこまない環境にあり、再生資源の一つである。深層水は、私たち人類が必要とする、エネルギー、肥料、各種金属類、水、ミネラル類など複数の重要資源を単一物として持っているユニークな資源である。さらに、深層水は、表層海水が数十～数千年低温・暗状態下におかれて形成され、熟成と清浄性が高く、安定した資源性といった利用上の優れた特徴をもつ。ここでは、深層水の資源利用の現状を紹介する。

2．海洋深層水資源の利活用

深層水は、飲食品・化粧品・入浴剤・医療治療補助剤などの様々な利用が工夫された。これまでに世界各地で開発された深層水商品の種類数は、1万点に達すると思われる。

飲食品・苦汁・塩・入浴剤・化粧品などは、深層水が含むミネラル類や塩が利用された。飲食品や化粧品では、深層水の清浄性も利用価値を高めている。また、海水のミネラル類は発酵促進作用が顕著で、複数種類の塩は飲食品の味を高める。飲料水と一部の化粧品では、清浄な深層水の脱塩水やミネラル類が使われている。深層水の含む有機物や、深層水中の微生物の抽出物の医薬品としての利用も始まった。

深層水の含む肥料を利用した海藻培養も進んでいる。また、深層水の低水温と清浄性を使って魚介類の蓄養や養殖も行われている。

深層水の低温は、建物冷房に利用され、70%以上の省エネ効果をあげている。熱帯・亜熱帯では、深層水の低温を利用した温帯野菜・果物・花卉栽培が可能で、生産物には温帯とは異なった付加価値がつく。海洋の表層温水と低温深層水の温度差を利用した発電も、商用段階に入ってきた。海水中にはリチウムを始め、ほとんどすべての有用金属類がとけていて、効果的な商業用の抽出技術の開発も近い（海水鉱山）。

3．21世紀社会を支える海洋深層水

深層水は、数十～数千年で再生し、人類が必要とするエネルギーと重要物質を含む巨大資源で、地下資源に代わる21世紀の人間社会を支える筆頭の基礎資源である。

資源利用にあたっては、汲み上げた深層水の資源を利用しつくし表層水と同じ性質にして表層に排水する必要があり、それには、深層水の資源を順番に多段利用することがポイントである。

S2. Supporting the 21st human society by deep ocean water resources

°Masayuki Takahashi
(President of the Deep Ocean Water Applications Society, Emeritus professor of the University of Tokyo and Kochi University)

1. Introduction

Material richness of individual person in human history has remarkably increased due to probable active applications of underground resources supported by the science and technology development. But two serious problems have been arisen, (1) possible exhaustion of underground resources and (2) environmental pollution caused by waste disposal. It is then critical to change from un-renewable underground resources to renewable ones.

Deep ocean water (DOW) below a depth of ca.200m where no enough light penetrates is a candidate for future renewable resource, which contains necessary essential resources such as energy, fertilizers, metals, water, and minerals as a single material. Furthermore DOW is aged, highly clean, and stable resource concentration formed under cold and dark condition over several 10 to 1,000 years. Here resource utilizations of DOW being developed will be introduced.

2. Resource utilization of DOW

DOW has been applied for various commercial goods including foods and drinks, cosmetics, bathing goods and supplements for medical treatments. It seems there have been up to 10,000 kinds of DOW commercial goods in the world. Foods and drinks, bittern, salts, bathing goods and cosmetics are applied minerals and salts as well as cleanliness of DOW. Drinks and some cosmetics use desalinated DOW and minerals of clean DOW. Some organic matter of DOW and materials extracted from micro-organism in DOW have now been applied for medical treatments.

Seaweeds are cultured using the fertilizers in DOW. Fish and shellfish have now been held and cultured using cold and clean DOW.

Cold DOW can be applied for building air conditioning at more than 70% savings of energy. Ocean thermal energy conversion (OTEC) is now in the stage for commercial operation. Metals including lithium in DOW will be commercially extracted from DOW in the near future.

3. DOW as the major resource for the 21st human society

DOW regenerates within several 10 to 1,000 years and is huge its resource as well as containing energy and materials essential for human being. DOW is probably a primay resource to support the 21st human society in place of un-renewable underground resources.

In DOW resource applications, DOW has to be returned to the sea after removal of resources in order to avoid environmental perturbation. This will be solved by applying resource utilization in series such as cascade or multi-steps.

S3. 海洋深層水産業の将来性

○浅川　良住（マリンゴールド株式会社）

１．はじめに

今回は深層水産業について経営者の立場で話したいと思います。弊社が海洋深層水ミネラルウオーターを最初に作ったときは“海水から塩を抜いた水が売れるか”　と陰口されたものです。しかし、海の水を飲む！ロマンがあると思いませんか？　海の水である目新しさをメディアが取り上げてくれたこともあり、発売当初はそれなりの売上を上げることが出来ました。しかしながら、いつかは飽きられると考え、この不思議議な水の効能を消費者に知らせるべく大学との共同研究に着手することにしました。高知大学医学部との共同研究では深層水中の有機質と無機質を分離することに成功し、動物実験で分子量が小さく分解が難しい難分解性有機質に抗動脈硬化作用があることを発見し国際シンポジウムで研究成果を発表しました。しかし、難分解性有機質の組成は無限大に近く、１大学と１企業とで手におえる研究ではなく、莫大な資金をかけた国家プロジェクトで行うべき研究であると実感した次第です。

２．新製品の開発

メーカの技術者にとって「新商品」を作ることはそれ程難しいことではないが、ヒット商品となると非常に難しい。また、商品の売れ行きを持続させるのも難しいことです。何故なら人間は飽きっぽくて忘れやすいからです。そのためには消費者に身体に良い商品であることをメディアを通じて定期的に知らせることが必要です。商品原価を抑えて宣伝効果を上げるために“テレビが取材してくれる商品とはどの様なものか？”記者に聞いたところ、一言で“３分以上のロマンのある物語を作れる商品であること”との返答でした。また、メーカにとって、薬事法のハードルをクリアーすることも必要です。現況としましては大学等により効能に関する研究論文が発表され、かつ、その研究成果が新聞等に掲載されることが薬事法による制約を回避しやすくする一策となっていることも事実です。新商品開発の中で最も重要なことはエビデンスの構築と理論武装です。これをやらずに病気に効く等の宣伝を行えば、皆さんもご存知のような“深層水はインチキ”などと言われ、１度悪評が立てばそれを覆すことは容易でなくなります。

余談になりますが、近年、我が国では大量のペットボトル（飲料水）を国内生産し、また、海外調達していますが、何故、大手飲料水メーカは海洋深層水の使用に目を付けないかとの意見があります。この意見に対して、大手飲料水メーカ出身の弊社技術顧問によれば　“世界的な飲料水メーカは初めてで、よく解らない水には手をださない。しかし、深層水ミネラルウォーターが出来て２０年が経過して健康被害が生じておらず、また、その資源量を考慮するに無関心な訳がない。将来を見据えて虎視眈々と狙っているのではなかろうか？”との意見もあります。

３．海洋深層水産業の衰退そして復活

深層水商品の売上高は年々落ちています。経営者にとって新商品開発において１番大事なことは売れて利益を得ることです。それでは深層水産業の将来をどうすればよいか？　海洋深層水利用学会の会報では毎年様々な分野での研究発表がなされ、バライロの産業になると思われてきました。しかし残念ながら一部の人しか知られていないのが現状です。“コップの中でやっている産業としか思えない”。そこで私のお願いは“メディアに取り上げてもらうことを前提とした研究発表を心がけてもらいたい。”ただし、基礎研究や実証試験、効果の検証がなされていない商品の宣伝はやめてもらいたい。京セラの稲盛会長の言うように、“正しいことをまじめにやる。それが成功の秘訣。”ペテンやインチキはやめ、薬事法のハードルをクリアーしましょう。

母なる海、海洋深層水産業で生きている我々は『清く、正しく、美しく生きようではありませんか！』

S3.Future prospect of deep ocean water industry

Yoshizumi Asakawa
(Marine Gold Co. Ltd.)

1. Introduction

I would like to speak on the deep ocean water (DOW) industry from the standpoint of a company manager. When my company produced mineral water from DOW, people backbit such as "No people will buy such water by simply extracting salts from seawater". However, don't you think it is romantic to drink water from the ocean? Due to publicity of the original idea of drinking water from the ocean by the mass media, the sales picked up at the start. I was further challenged to find out possible effects of DOW on human health together with some university specialists. With the cooperative research of the medical school of Kochi University, we succeeded in separating organic and inorganic substances in DOW, and found that small persistent organic molecular materials had anti-hardening effects on the arteries. The findings were presented at an international symposium. Unfortunately, further research was extremely difficult because of fairly complex composition of the persistent organic matters.

2. Development of a new product

Although it is not so difficult to develop new products, it is hard to maintain the popularity of a product. It is also hard to sustain the sales of a popular product in the market, because people tend to get tired and to forget easily. It is therefore important to constantly remind the people through the mass media that the product is good for human health. One day, a TV producer suggested to me that a product should ideally have a romantic story of more than 3 minutes for TV. It is also essential for makers to comply with legal regulations such as the Drugs, Cosmetics and Medical Instruments Act. It also helps such compliance by publishing research papers on the positive effects by research specialists such as university staffs and being played up by mass media. The most important for developing a new product is to show the evidence and its logical interpretation and application. Without spending enough efforts such as those mentioned above, someone publicizing any positive health effects will be branded immediately as trickery. Such a bad reputation is extremely difficult to correct.

3. Decline of the DOW industry and its recovery

Sales of DOW products have decreased year after year in Japan. The most important consideration for company managers in developing new products is to make profits in sales. What we should do for the future of the DOW industry? Although many researches on DOW resource applications have been reported annually through the DOWAS, they are only popular and known within a limited group of people. This is quite unfortunate. My desire is encouraging more researches on DOW applications and attracting the mass media. It is also necessary to firmly stop or forbid any advertisement of products whose effects have not been proven with basic research and actual experiments.

S4. 從土壤礦物質專家到海洋礦物質專家
台肥集團海洋深層水事業之發展與願景

李 復興（台灣肥料股份有限公司董事長）

一、從土壤到海洋

台灣肥料股份有限公司為 1946 年當時中華民國經濟部為配合政府的農業政策所創設的國營事業之一，主要業務為生產農業用肥料。現在台肥公司為台灣最具規模之肥料生產事業體，持續提供國內農業用肥料，每年除了生產 80 餘萬噸農業用肥料之外，並進口轉售尿素、氯化鉀、硝酸銨鈣等等供應國內市場所需，更著手參入進口化工產品、電子級化學品及生技產品之生產、開發或進口供銷國內外市場所需. 1999 年在政府積極推動公營事業之民營政策下，台灣肥料公司正式轉為民營事業，並成為一家股票上市之公司。7 年後的 2006 年，台肥成立 60 周年時，為推動企業轉型，利用花蓮工廠轉投資成立台灣海洋深層水股份有限公司，並在 2012 年成為台肥集團百分之百獨資之子公司。從過去集中於土壤、農業之事業主體，台肥集團開始著手發展海洋事業，期待可打造全新的事業版圖。

二、台肥集團海洋深層水事業的展開

海洋深層水因為其潔淨、礦物質均衡特性被譽為新興的水資源，然而因為受限於地理條件，在全球只有 4 個國家進行產業化利用，分別為美國(夏威夷)、日本、韓國及台灣，其中又以台灣因位於熱帶及亞熱帶地區擁有較溫暖之氣候，除在食品、飲料、農業、水產之利用外，冷能之商業化運用亦為國內外矚目的焦點之一。台肥集團為發展海洋深層水事業，在 2004 年董事會審議通過「花蓮廠海洋深層水投資計畫」，選定面向太平洋之花蓮廠為基地成立台肥海洋深層水園區。本基地位於花蓮縣中部七星潭最南端，並鄰近花蓮港，占地約 46 公頃，內容包括取水工程、水處理設施、包裝飲用水、飲料工廠及體驗、度假園區等計畫。園區內海洋深層水取水工程於 2007 年完成，自七星潭水深 662 公尺處，以長達 4,950 公尺，內徑 20 公分之高密度聚乙烯(HDPE)管線，汲取海洋深層水至陸地上進行利用。下列三階段目標進行園區開發：

1. 完成產品生產線與觀光工廠建設：建構深層海水取水設施、水處理設施、產品生產線，致力於可上市產品之開發與生產，最終將工廠成為具備觀光教育功能之場域。事業初期開始便積極與國內各大學研究機構，合作展開深層海水產品之基礎機能研究，並以研究成果落實商品化開發。
2. 建構休閒體驗設施：此階段為自製造業應用升級至服務業應用的第一步，除了將拓展既有園區建設特色餐飲食堂、結合地域特色之商業販賣及海洋深層水足療體驗等設施，做為產業應用示範點，期待可開始吸引觀光客來訪。
3. 大型度假飯店：結合地方元素及海洋深層水之應用，打造擁有 250 間面海客房的六星級觀光度假飯店，並提供深層海水之理療 SPA 服務，展開大規模事業利用，預計 2017 年底前可完成所有開發。

三、未來發展願景

台肥集團自過去作為土壤礦物質專家以肥料照顧土壤的健康，進而以被譽為是上帝的配方的海洋深層水關照人類的健康。在海洋深層水事業中，台肥集團除持續穩固相關基礎研究以協助台灣成為未來相關研究之基地外，再者期待藉由利用海洋深層水打造世界級養生渡假園區，作為全球深層海水產業利用發展之示範發信地，以推動海洋深層水之產業利用。

S4. A Transition From a Soil Minerals Expert to Ocean Minerals Expert: The Vision of Taiwan Fertilizer Co., Ltd. for Deep Sea Water Development

Fu-Hsing Lee (Chariman of Taiwan Fertilizer Co. Ltd.)

I. From Soil to Ocean

In 1946, Taiwan Fertilizer Co., Ltd. (TFC) was established as a state-owned enterprise according to the agricultural policy implemented by the Ministry of Economic Affairs. TFC has primarily focused on producing agricultural fertilizers. As the largest national business entity in fertilizer production, TFC currently produces more than 800,000 tons of agricultural fertilizers annually for domestic industries. The company imports and sells chemical products (e.g., urea, potassium chloride, and calcium ammonium nitrate) to satisfy demand in the domestic market. TFC is also involved with the production, development, and importation of chemical products, electronic chemicals, and biotechnology products to supply the needs of both international and domestic markets. In response to the privatization policy regarding public enterprises enacted by the government in 1999, TFC officially became a private enterprise and was listed on the Taiwan Stock Exchange. On the company's 60th anniversary in 2006, TFC promoted its transformed status by investing in a plant in Hualien to establish the deep sea water (DSW) company, Taiwan Yes, which became TFC's sole-owner subsidiary company in 2012. Hoping to develop within a new industry, TFC has transitioned from being only a soil and agricultural company to an ocean business entity.

II. The Beginning of the DSW Business

DSW is a water resource that has attracted attention recently, and is famous for purity and balanced mineral content. However, because of geographical limitations, DSW is restricted for industrial use to only four countries: the United States (Hawaii), Japan, South Korea, and Taiwan. Because Taiwan is situated in a warm tropical and subtropical zone, DSW is widely used in the food and beverages industry, agricultural industry, and aquatic products. Specifically, the commercial application of DSW in cold energy systems has received substantial global attention. In 2004, the proposal to investing in DSW in Hualien was approved at a board of directors meeting. Subsequently, TFC established the DSW plant and park (Taifer DSW Park, TDP) in Hualien, which faces the Pacific Ocean. This park, with an area of 46 ha, is located at the southernmost end of the Qixintan Area in Central Hualien and is near to the Hualien Harbor. The park is under development and will entail numerous facilities, including those intended for water treatment, drinking-water packaging, beverage production, and an exhibition area and holiday resort. The DSW abstraction facility was completed in 2007, extracting DSW for land use by employing a high-density polyethylene (HDPE) pipe that is 4,950 m in length and 20 cm in diameter. This pipe was installed at a depth of 662 m in the ocean near Qixintan. TDP is currently being developed based on the following three-stage goal:

1. Complete the establishment of a production line and exhibition center for tourists by (a) constructing a DSW abstraction facility, water-treatment facility, and production line, (b) developing and manufacturing products that can be sold on the market, and (c) converting the plant park into a tourist attraction and educational facility. At the initial stage of park development, TDP has been actively cooperating with domestic university research institutions in conducting basic research regarding DOW products and commercial development based on the findings.
2. Construct a leisure facility: This stage marks the first step to converting TFC from a manufacturing industry into a service industry. The park will serve as a tourist attraction area by incorporating exotic restaurants, stores that sell regional products, and a DOW spa treatment facility that will serve as an industrial application demonstration center.
3. Establish a large-scale six-star holiday resort hotel that will be integrated with local and DSW elements and provide 250 ocean-view hotel rooms and DSW spa services. By further expanding DSW business, this goal is expected to be completed by the end of 2017.

III. Future Vision

In addition to being an expert in enriching soil by using mineral fertilizers, TFC is now a company that vitalizes human health using DSW. As a DSW business unit, TFC is not only consolidating relevant DSW research to further transform Taiwan into the global leader of DSW research, but is also employing DSW to create a world-class health resort that will also serve as a demonstration center to inspire the global development of the DSW industry and promote the use of DSW in other industries.

一般講演１
〈海洋・水質関連／生物・水産関連１〉
Session 1: Water Quality, Biology and Fisheries 1

座長　　今田　千秋

（東京海洋大学　大学院　海洋科学技術研究科　教授）

Chairman: Chiaki Imada

(Graduate School of Marine Science and Technology,
Tokyo University of Marine Science and Technology, Professor)

1. 台灣深層海水長期水質監測

°劉志恆、徐雅玲、陳建宇、黃秉益(石資中心)

1. 前言：

深層海水資源之應用性廣泛，台灣國內已有多數廠商投入相關產品之開發。為提供水源安全性依據以建立消費者信賴及作為相關產品開發之基礎。財團法人石材暨資源產業研究發展中心(石資中心)自2008年以來，針對台灣東部地區深層海水取水設施進行水質長期監測，並將監測結果建立全台灣最完整之深層海水水質資料庫。本研究即針對5年來檢測結果進行整理報告。

2. 實驗方法：

台灣現有4個可以穩定供水之取水設施，分布於花蓮縣及台東縣內。自2008年1月起，石資中心深層海水實驗室定期前往4取水設施採取深層海水樣品進行水質檢測。檢測項目如表2所示，分為一般項目、營養鹽、主要元素、微量元素、有害物質等六大類。所有檢測結果同步公告於深層海水知識服務平台，網址如下：http://www.dow.org.tw/water.php。

3. 結果與討論：

各取水設施之水溫分別為，HN:平均約10.6℃、HM:13.1℃、HS: 10.0℃、TN: 10.4℃，終年變動範圍為水溫±0.5 ℃、pH±0.2、鹽度±0.4 psu，未見季節性變化。營養鹽部分，磷酸鹽約2.4 μM，矽酸鹽75.0 μM，與表層海水相較，確實具有豐富營養鹽，但與其他國家同等取水深度之深層海水比較，則無太大差異。水中未有大腸桿菌群、總菌落數檢出；葉綠素a皆低於偵測極限(0.03 mg/L)。主要元素檢測結果：鈉含量平均約11,600 mg/L、鉀及鈣420 mg/L、鎂1,380 mg/L、溴69.5 mg/L、硼4.2 mg/L、氟0.9 mg/L、硫酸根2,610 mg/L。有害物質之農藥類、重金屬等皆未檢出外，自2011年5月福島核災後開始的放射性核種亦皆於法定標準以內。

綜合以上結果，台灣東部取得之深層海水與其他國家相較之下，同樣具有低溫、性質安定、富營養及礦物質均衡及潔淨之特性，是可以多元化利用的資源。特別是台灣因位處熱帶及亞熱帶地區，對冷能運用有絕大之優勢，應更強化此領域之應用。

表1 深層海水採樣地點及其取水深度

採樣地點	取水深度(m)
HN(花蓮七星潭北)	710
HM(花蓮七星潭中)	618
HS(花蓮七星潭南)	662
TN(台東知本溪北)	637

表2 水質檢測項目

一般項目	水溫、pH、鹽度、總有機碳
營養鹽	硝酸鹽、磷酸鹽、矽酸鹽、亞硝酸鹽、氨氮
主要元素	鈉、鉀、鈣、鎂、氯、溴、鍶、硼、氟、硫酸鹽
微量元素	鐵、鋅、銅、鉛、錳、鎳、銀、鉬、釩、銻、砷
微生物	大腸桿菌群、總菌落數、葉綠素a
有害物質	有機氯農藥、有機磷農藥、氨基甲酸鹽農藥、重金屬、放射性核種

註：檢測方法係依據標準檢驗局公告之CNS方法，以及環保署公告之NIEA方法。

1. Study of long-term water quality monitoring of deep sea water in eastern Taiwan

°Zhi-Heng Liu, Ya-Ling Hsu, Jian-Yu Chen and Ping-Yi Huang
(Stone and Resources Industry R&D Center, Taiwan)

I. Introduction

Deep sea water (DSW) has a wide range of applications today. Many companies have already started developing relevant products in Taiwan. The Stone and Resource Industry R&D Center (SRDC) has been conducting long-term DSW quality monitoring since 2008 to establish a guideline of water safety and to create the consumer's support that is required for business development. All results were submitted to a comprehensive water quality database in Taiwan. This report presents the database results for the past 5 years.

II. Methods:

Currently, 4 intake facilities, located in Hualien County and Taitung County, provide reliable sources for DSW samples in Taiwan (Table 1). These facilities have periodically conducted DSW sampling since 2008, and the DSW Laboratory of the SRDC immediately analyzed the samples. The indicators for water quality analysis are presented in Table 2. Six categories, including general indicators, nutrients, major elements, trace elements, and harmful materials are analyzed using National Institute of Environment Analysis (NIEA) and National Standards of Republic of China (CNS) standard methods. The results are presented on the DSW Knowledge Service Platform (http://www.dow.org.tw/water.php).

III. Results and Discussion

The average water temperatures were 10.6 °C at HN, 13.1 °C at HM, 10.0 °C at HS, and 10.4 °C at TN. Seasonal changes were as low as ± 0.5 °C for temperature, ± 0.2 for pH, and ± 0.4 psu for salinity. The results for the nutrient indicators, a concentration of nitrate and silicate, were approximately 2.4 μM and 75.0 μM, respectively. DSW samples taken from Eastern Taiwan had richer nutrients compared with surface sea water and had characteristics similar to those of samples from facilities in other countries where the intake depth was comparable. No coliforms or total bacterial count were detected, and chlorophyll *a* was below the detection limit (< 0.03 μg/L). The results for major elements are presented as follows: Na, 11,600 mg/L; K and Ca, 420 mg/L; Mg, 1,380 mg/L; Br, 69.5 mg/L; B, 4.2 mg/L; Cl, 0.9 mg/L; and SO_4^{2-}, 2,610 mg/L. Harmful materials, such as pesticides and heavy metals, were not detected. Radionuclides were within the legal standard, which was set after the Fukushima nuclear disaster in May 2011.

Based on the results of this study, DSW in Eastern Taiwan is cold, stable, nutrient-rich, balanced in minerals, and clean, similar to that in other countries. DSW is a resource that has diverse applications. Moreover, Taiwan's tropical and subtropical location provides a major advantage in low-temperature energy use, an application that should be further developed.

Table 1 Deep sea water sampling location and depth

Sampling Station	Location	Intake depth (m)
HN	Northern Ci-Sing-Tan bay in Hualien	710
HM	Central Ci-Sing-Tan bay in Hualien	618
HS	Southern Ci-Sing-Tan bay in Hualien	662
TN	Northern Chiben Stream in Taitung	637

Table 2 Indicators of water quality analysis

General indicators	Temperature, pH, Salinity, Total Organic Carbon
Nutrient	NO_3, PO_4, SiO_2, NO_2, NH_4
Major Elements	Na, K, Ca, Mg, Cl, Br, Sr, B, F, SO_4
Trace Elements	Fe, Zn, Cu, Pb, Mn, Ne, Ag, Mo, V, Sb, As
Microorganisms	Coliforms, Total Bacterial Count, Chlorophyll *a*
Harmful materials	Organochlorine Pesticides, Organophosphorus Pesticides, Carbamate Pesticides, Heavy metals, Radionuclides

*Testing is done in accordance with CNS of BSMI and NIEA of EPA.

2. 人工海底山脈の現状と課題

○鈴木達雄（株式会社　人工海底山脈研究所）

１．はじめに

日本では沿岸の基礎生産を増加させ水産資源の回復を促進するため、海底に人工の山脈を構築する公共事業が行われている。海の真光層以深の海水に豊富に含まれる栄養塩類を自然の潮流を利用して人工的に真光層に混合するのが目的である。この事業のきっかけは1969年にRytherが発表した論文で、世界の魚類生産の 50％は、全海洋面積の僅か0.1％の深層水が湧昇する海域でされているという衝撃的な事実であった。

人工的に湧昇海域をつくれないか、様々な机上研究を経て1995年に人工海底山脈の実証事業が行われた。栄養塩類の湧昇により基礎生産が増加し、標本船による対象海域の漁獲量が事業前の6倍に増加し、人工衛星による海色の観測から周辺海域のクロロフィルa濃度が1.5倍に増加した。たんぱく質の60％を水産物に頼る日本で、深層水を利用した水産資源の増産は重要なテーマである。

２．人工海底山脈事業の現状

日本政府は前記実証事業の成果に基づいて2002年から県を事業主体とするこの公共事業に補助金を交付し、既に11海域で人工海底山脈事業を実施し大きな成果を得ている。これらの実績を基に水産庁は、2010 年に人工海底山脈を直轄事業に昇格させ、日本の排他的経済水域において大規模に進めている。

３．今後の課題と展望

海洋には気候変動、海水温の上昇、海流の変動などの大きな自然環境の揺らぎがある。自然地形に比較すれば微細な人工構造物による影響を評価するためには高精度の技術が必要である。中でも海洋観測、流動解析、施工における技術には課題が残っている。

海洋観測では、詳細な流向・流速、密度、水質の把握のほか、人工海底山脈で発生する内部波と鉛直混合のつながりを究明することが重要な課題となっている。また、リモートセンシングによるクロロフィル a 濃度分布の解析手法の高度化が求められている。

流動解析では、密度成層流下の鉛直混合を表すために浮力を考慮した精度の高いモデルが必要であり、人工海底山脈の背後に生じる大規模な渦の非定常運動の解析に適した乱流モデルが必要である。また、適切な流動解析モデルと観測結果との整合を図ることにより、栄養塩類の真光層への添加量を適正に評価する手法を開発する必要がある。

施工技術では、設置海域の大水深化による事業規模の拡大に伴い効率の高い人工海底山脈を構築する技術が求められる。海底山脈による湧昇効果や反流域の面積は流れに対する方向の投影面積に影響を受けるが、流れに直交する方向の投影面積には殆ど影響を受けない。そこで、より少ない体積の人工海底山脈で同等以上の湧昇効果と魚礁効果を得る提案がある。人工海底山脈を構築する際に海面から投入するブロックの散乱を制御することで流れに対する投影面積を変えずに流れに直交する投影面積を減少させる。この実現のために複数のブロックをロープやワイヤでルーズに連結して投下することでブロックの散乱を大きく制御する技術が開発された。

2. Present status and tasks for artificial under-seamount

○Tatsuo SUZUKI (Artificial Sea-mount Institute)

1. Introduction

Artificial under-seamounts were built as a public enterprise for increasing fisheries resources by enhancing primary productivity. This is due to mixing of rich nutrients in the bottom water to the euphotic zone and helped by tidal current. This challenge followed Ryther's classic paper (1969) suggesting that 50% of world fish is produced in the upwelling area occupying only 0.1% of the world ocean.

After long term preparations, a public enterprise constructing an under-seamount was undertaken in 1995. The under-seamount enhanced primary productivity, increased the satellite detected surface chlorophyll *a* 1.5 times in the area, and increased fish catch 6 times. Ocean fertilization is an important subject because 60% of total protein is supplied from fisheries products in Japan.

2. Present status of under-seamount

According to the successful results mentioned above, the Japanese government has subsidized prefectural governments to construct artificial under-seamount since 2002, and constructions were carried out in 11 different sea areas with successful results. Then, the Fisheries Agency of Japan started construction of under-seamounts in the exclusive economic zone under their direct control after 2010.

3. Future tasks and prospects

Since there are natural fluctuations in various parameters in the ocean, highly sensitive measurements are required for detecting possible effects of a relatively small man-made structure.

In ocean monitoring, flow direction, flow speed, density and quality of water mass are essential in detail measurements. It is also important to find out possible vertical mixing of water associated with internal waves created by under-seamount. Chlorophyll determination by satellite is also important for further improvement.

In flow analysis, a precise model concerning buoyancy is necessary for describing vertical mixing of water under density stratified water column, and turbulent flow model is also requested for analyzing irregular motion of large vortices formed behind the under-seamount. Furthermore a procedure for predicting nutrients supplied to the euphotic zone is necessary using a suitable flow dynamic model and ocean monitoring data.

For construction technology, efficient technology development is required in greater depths. The small number of artificial under-seamount is essential for both upwelling and fish attracting effects. For minimizing dispersal of concrete blocks, several blocks loosely connected together was developed for dumping.

3. 探討深層海水對蛹蟲草液態與固態發酵之菌絲體生長及功效成分生成之影響

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1. 前言：

在中國傳統醫藥中，蛹蟲草 (*Cordyceps militarys*) 佔有相當重要的地位，具有藥用與食用之雙重價值。過去許多研究指出 *C. militaris* 具有抗氧化、抗發炎及抗腫瘤等多種功效，而其活性成分包含腺苷 (adenosine)、β-1,3-葡聚醣 (β-1,3-glucan)、蟲草素 (cordycepin) 等。

深層海水 (Deep sea water, DSW) 中含有豐富的無機鹽類與微量元素，包含 Mg^{2+}、Ca^{2+} 與 K^{+} 等，根據過去研究發現，DSW 可促進紅麴菌生長及功效成分 monascin 與 ankaflavin 生成。在樟芝液態發酵方面，DSW 亦可促進菌絲體生成，提高 triterpenoids 與 polysaccharide 含量。

根據豐富的無機鹽類與微量元素且能促進真菌生長及代謝物生成的特性，本研究利用 DSW 進行 *C. militaris* 液態與固態培養，並進一步探討 DSW 及其離子對功效成分生成之影響。

2. 實驗方法：

本研究以蛹蟲草菌株 *Cordyceps militaris* BCRC 32219 為實驗菌株

C. militaris 培養於 YM 平板培養基，在 24°C 下培養 10 天，並接種於種菌培養基中，以 24°C、120 rpm 振盪培養 5 天後進行液態與固態培養。

液態培養是將 *C. militaris* 接種於不同濃度之 DSW (0X、5X、10X、15X 及 20X) 液態培養基中，在 24°C、120 rpm 下振盪培養。固態發酵是將 *C. militaris* 接種於不同濃度之 DSW (0X、10X、20X 及 30X) 固態基質中，例如紅薏仁、糙米及糙燕麥，並在 24°C 下培養。

為了解離子在 *C. militaris* 發酵過程中所扮演的角色，分別添加與 10X DSW 同濃度之 Mg^{2+}、Na^{+}、K^{+}、Ca^{2+}、Se^{6+}、Fe^{3+} 等 6 種離子溶液至固態基質中。

3. 結果與討論：

結果顯示，在液態發酵部分，DSW 能促進 *C. militaris* 菌絲體生長，並呈現劑量效應。以 20X DSW 培養 *C. militaris* (0.765g) 其菌體乾重顯著高於以超純水 (Ultra-pure water, UPW) 培養之 *C. militaris* (0.692g)。在固態發酵方面，*C. militaris* 發酵之糙燕麥中 adenosine、cordycepin 含量均顯著高於其他基質。此外，cordyceoin 之生成隨著 DSW 濃度提高而增加。30X DSW 可提高 cordycepin 含量達到 16.79 mg/g，顯著高於 UPW 組。然而，DSW 中的 Mg^{2+}、Ca^{2+} 和 Fe^{3+} 可能是造成 *C. militaris* 發酵糙燕麥中 adenosine 與 cordycepin 含量提升之主要離子。

3. Effects of Deep Seawater on the Mycelia Growth and Functional Ingredients Formation of *Cordyceps militaris* under Submerged and Solid Fermentation

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I. Introduction

In traditional Chinese medicine, *Cordyceps militaris* occupies a very important position for both medical and edible purposes. Many previous studies indicated that *C. militaris* has antioxidative, anti-inflammatory, anti-tumor, and other properties due to active ingredients such as adenosine, β-1,3-glucan, and cordycepin.

The deep seawater (DSW) contains rich metals and trace elements including magnesium (Mg), calcium (Ca), potassium (K), etc. According to previous studies, DSW was proven to promote *Monascus purpureus* growth and functional compounds monascin and ankaflavin formation. In submerged fermentation of *Antrodia camphorata*, DSW also promoted mycelia growth and raised triterpenoid and polysaccharide levels.

With the rich metals and trace elements able to stimulate the growth or metabolite production of fungi, this study used DSW to submerged and solid culture of *C. militaris*, and further investigated the effect of DSW and its ions on the functional ingredients formation.

II. Materials and Methods

Cordyceps militaris BCRC 32219 was used as the experimental strain in this study.

C. militaris was cultured in YM medium plate at 24°C for 10 d and then cultured in the seed culture medium at 24°C with 120 rpm for 5 d before submerged and solid culture.

In the submerged culture, *C. militaris* was cultured in different concentration of DSW (0X, 5X, 10X, 15X, and 20X) at 24°C with 120 rpm. In the solid culture, *C. militaris* was cultured in solid substrate such as red pearl barley, brown rice, and oats including different concentration of DSW (0X, 10X, 20X, and 30X) at 24°C.

The ion solution of magnesium (Mg), sodium (Na), potassium (K), calcium (Ca), selenium (Se), and iron (Fe) was respectively added in to the solid substrate with equal concentration to 10X DSW in order to understand the role of ion on the fermentation of *C. militaris*.

III. Results and Discussion

The result showed that in submerged fermentation, DSW promoted the mycelia growth of *C. militaris* with dose- dependent response. The biomass of *C. militaris* cultured in 20X DSW (0.765 g) was significantly higher that cultured in ultra-pure water (UPW) (0.692 g). In solid fermentation, *C. militaris* produced more adenosine and cordycepin contents in oats than other substrates. Furthermore, cordycepin formation was increased with rising DSW concentration. Thirty-folds of DSW up-regulated cordycepin produced on to 16.79 mg/g, and which was significantly higher than UPW group. However, Mg, Ca, Fe may be the functional ions in DSW for enhancing adenosine and cordycepin levels of *C. militaris*-fermented oats.

4. 層別定量サンプリングによる相模灘深層の動物プランクトン研究

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1．はじめに

「相模灘」は、日本の本州中央部の神奈川県および静岡県の沿岸に位置し、狭義の「相模湾」を含み、伊豆半島、伊豆大島および三浦半島の南端で囲まれる区域を指す。海底地形は図１に示すように複雑で、相模トラフが伊豆・小笠原海溝から分岐するように伸びて海域南東部から中央部に向けて入り込み、谷を形成している。最大水深は 1500 m 以上となっている。東京海洋大学浮遊生物学研究室では、この海域の水深 1500 m の定点において動物プランクトンの層別定量採集を行い、海洋構造と生物分布との関係を調べている。本発表ではその結果の一部を紹介し、深層水研究における学際的取組の意義を強調したい。

2. 材料と方法

相模灘の定点 S3（35° 00’N, 135° 20’E）（図１）において、東京海洋大学の研究練習船「青鷹丸」(167 ton) により、動物プランクトンの層別定量サンプリングが行われている。サンプリングには、初期には MTD ネット (Motoda, 1971)、近年では IONESS（喜多村ほか, 2001）が用いられている。MTD では海面から深度 1000 m まで、IONESS では深度 1400 m までの複数層で、水平曳きが行われた。ネットの目幅は 0.33 mm あるいは 0.2 mm である。一連のサンプリングの前後には、CTD（クロロフィル蛍光計と光束透過率計付き）による鉛直プロファイルの計測が行われている。さらに IONESS は、網口に取り付けられた CTD で、曳網と並行して水圧(深度)、水温および塩分を記録している。

3. 結果の例、および今後の課題

この定点では、海面から数十 m までは高温で低塩分の沿岸水、そこから 200-300 m までは高塩分の黒潮を起源とする水が認められる。さらに特徴的な構造として北西太平洋の亜寒帯海域を起源とする低温・低塩分の水塊が、深度 400-600 m に貫入している。動物プランクトンの鉛直分布は、このような水塊の鉛直構造と対応した様相を呈している。特徴的な例として、北太平洋亜寒帯海域で優占する大型のカイアシ類 *Neocalanus* 属が、中層の低温・低塩分水が明瞭なとき、高い密度で出現している。面白いことに実は、歴史的には相模灘の中層にこの *Neocalanus* 属が分布する事実をまず海洋生物学者が発見（Omori, 1967）し、亜寒帯起源の水塊の貫入の存在が推測され、後に海洋物理学者らによってその貫入が確かめられたのだった。

生物の調査は、観測が容易ではなく、さらに分析に多大な労力と時間を要するため、時空間的に離散的なデータしか得られないのが実情である。しかし、上記の例が示すように、海は、学術分野の枠に囚われず総合的な視点で眺める者にその実態を垣間見せてくれるようである。ここに、学際的研究の重要性が改めて強調される。とくに、データが十分ではない生物調査の省力化、自動化、高速化が急務の一つであると考える。

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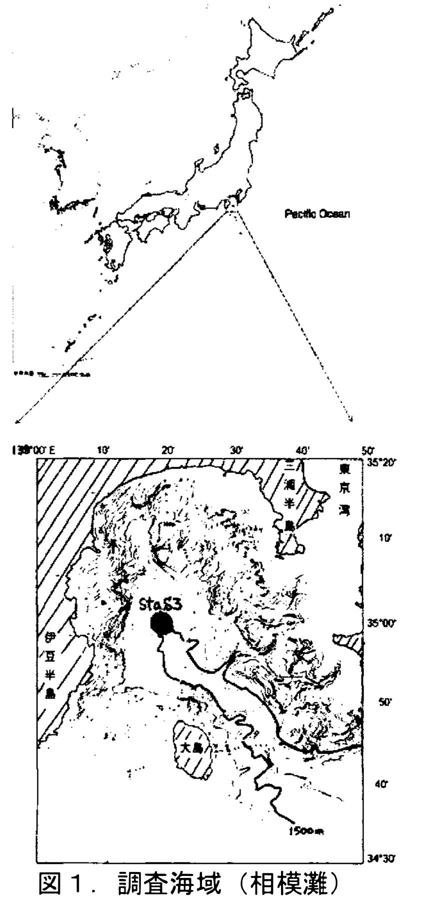

図１．調査海域（相模灘）
太線は 1500 m 等深線

4. Zooplankton study of Sagami Sea deep-water via quantitative stratified sampling

○Yuji Tanaka
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1. Introduction

"Sagami Sea" is located off Kanagawa and Shizuoka Prefectures at the central part of the Pacific coast of Honshu, Japan. This area is delimited by the southernmost end of Izu Peninsula, Izu-Oshima Is. and Miura Peninsula ("Sagami Bay" in a narrow sense is included in this "Sagami Sea"). The bottom topography is complicated (Fig. 1). Sagami Trough, branching from Izu-Ogasawara Trench extends from SE towards the central part of Sagami Sea, forming a valley. The maximum depth exceeds 1500 m. From the observation station at a depth of 1500 m, the Plankton Lab of Tokyo University of Marine Science and Technology (TUMSAT) is looking into the relation between the oceanographic structure and the distribution of marine organisms via quantitative stratified samplings. Here, a part of the observation results will be shown to stress the importance of interdisciplinary study in the deep-water science.

2. Materials and method

At Station S3 (35°00'N, 135°20'E, 1500 m-deep) in Sagami Sea (Fig. 1), depth stratified zooplankton samplings have been done via *Research and Training Vessel Seiyo Maru* of TUMSAT, by use of MTD net (Motoda, 1971) or IONESS (Kitamura et al., 2001). MTD and IONESS were horizontally towed at several layers from 0 to 1000 m and 0 to 1400 m, respectively. Plankton nets with mesh opening of 0.33 mm or 0.2 mm were used. To observe the vertical structure of the water column, a CTD with a chlorophyll-fluorometer and a transmissometer was deployed before or after a set of samplings. IONESS is also equipped with a CTD at the mouth-frame of the net.

3. Results, and future issue

In this station, the surface layer is occupied by warm and less-saline coastal water from the surface down to a few tens of meters, followed by warm and saline water originating from the Kuroshio. As a typical structure, cold and less saline water from the subarctic zone penetrates into a layer at 400-600 m. It seems that the vertical distribution of zooplankton here corresponds to the layered structure of the water column. A representative example is seen in large copepods (*Neocalanus*) appearing abundantly in the 400-600 m layer when the mid-water intrusion is clearly seen. Historically, it was a biological oceanographer (Omori, 1967) who first found the existence of *Neocalanus* in the mid-layer of Sagami Sea and assumed that in Sagami Sea there must be subarctic water intrusion. This assumption was later confirmed by physical oceanographers.

As to biological oceanographic investigations, because observing or capturing organisms in the field is not easy in the first place, later analyses require some labor and time. This has been limiting the biological oceanographic data set to be fairly discontinuous both spatially and temporally. Nevertheless, as is exemplified here, it seems we can uncover the ocean by tackling holistically. In this context, the importance of interdisciplinary studies can be stressed. One thing we need to focus may be to establish the means to save labor in biological oceanographic survey by automatizing and speeding.

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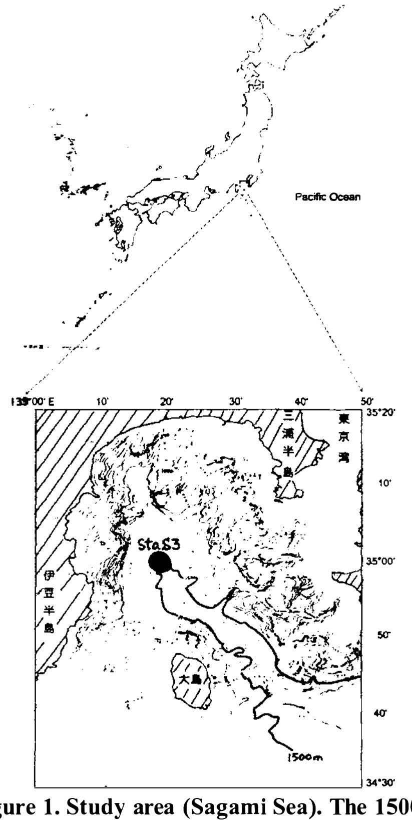

Figure 1. Study area (Sagami Sea). The 1500 m isobaths are drawn with a thick line.

5. 深層海水培育微藻之油脂特性分析

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1. 前言：

深層海水一般指海水深度 200 公尺以下，長年無陽光照射，水質幾乎不受到外在環境影響之海水，具有豐富的礦物質及營養鹽、低溫潔淨等特性，因此被廣泛應用於醫藥、化妝品、食品飲料、水產養殖、農產品培植或冷凍空調等產業。微藻乃單細胞生物，部分藻株的油脂含量高、培養時間短，可作為生產生質柴油的油脂來源。尤其微藻培養過程不佔用農耕地，甚至可利用沙漠、湖泊、海洋大量培養，且可利用其生長過程吸收二氧化碳以進行光合作用產生油脂的特性，將發電廠或煉油廠排放之二氧化碳吸收固定，因此是相當具有發展潛力的生質燃料料源。

本研究擬分別利用深層海水與表層海水培養微藻，以探討深層海水富含營養鹽、潔淨無污染等特性對於微藻生長速率、微藻生物量及油脂特性的影響，並評估應用深層海水生產微藻生質柴油之發展潛力。

2. 實驗方法：

本實驗所使用之深層海水為水深 662 公尺之海水，由台肥公司花蓮廠提供。表層海水取自國立台灣海洋大學基隆外海。本實驗所培養的牟氏角毛藻(*Chaetoceros muelleri*) 購自東港水試所。添加 Walne's medium 作為培養微藻過程之營養鹽。牟氏角毛藻放入裝滿深層海水或表層海水的培養瓶中培養，海水溫度維持在 25-26 ℃，並配合光照強度 11000-12000Lux 的紫外光照射藻水，另利用空氣泵浦將空氣打入培養瓶中除提供碳源外也攪動微藻體，以利微藻生長。

利用分光光度計測得微藻種的特徵波長，得到不同藻水濃度的吸光值(OD)，建立 OD 值及藻重關係之線性檢量線，藉以觀測不同培養時間的微藻生物量。利用離心收集之微藻體以超音波破碎藻胞壁，利用氯仿和甲醇溶劑萃取出粗油脂，可進行其油脂重量及組成分析，再經轉酯化反應得到脂肪酸甲酯即為微藻生質柴油。可使用氣體層析儀搭配火焰離子化偵測器分析其脂肪酸結構；再配合油品分析儀器分析其熱值、殘碳量、元素分析等。

3. 結果與討論

牟氏角毛藻經由分光光度計得到特徵波長為 681nm，再由牟氏角毛藻體乾重配合微藻水濃度所得之吸光值，建立一線性檢量線關係式為 Biomass $y=0.583x+0.009$，決定系數 $R^2=0.993$。培養於深層或表層海水的牟氏角毛藻生物量隨培養天數增加有明顯提高之趨勢，如圖 1 所示，尤其利用深層海水所培養之牟氏角毛藻生物量於第 11 天時到達 292mg/L，遠大於以表層海水所培養之生物量 178mg/L，可能因為深層海水含有大量矽酸鹽可促進微藻之增殖。

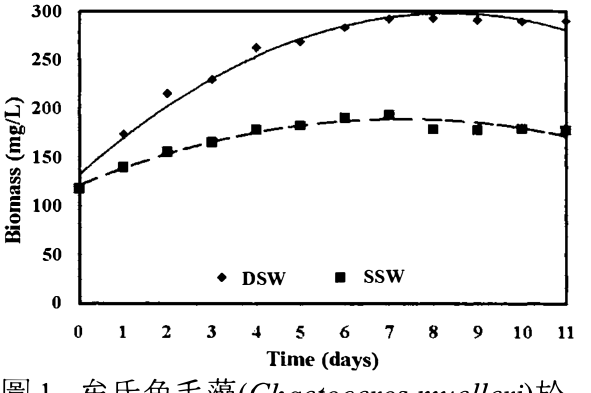

圖 1. 牟氏角毛藻(*Chaetoceros muelleri*)於不同海水中的生物量變化

5. Analysis of Microalgae Lipid Cultured in Deep Sea Water

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I. Introduction

Deep seawater (DSW) which generally refers to seawater below 200 m of sea level contains rich inorganic minerals and nutrients, and has excellent characteristics of low temperature, poor bacteria, and stable water properties. DSW has been widely applied in pharmaceutics, cosmetics, aquaculture, energy, and air conditioning, etc.

Microalgae can be grown in deserts, lakes, or oceans. Carbon dioxide emitted from fossil fuel-fired power plants can be absorbed and fixed by microalgae during photosynthesis facilitating lipid growth. DSW is considered in this study for culturing microalgae in order to investigate its effect on the growth rate, biomass quantity, lipid content, and biodiesel characteristics of microalgae. The potential of using DSW for microalgae biodiesel production is evaluated as well.

II. Experimental Details

DSW used in this study was provided by the Hua-Lien Factory of Taiwan Fertilizer Co. Ltd. The surface seawater (SSW) was taken from outside the National Taiwan Ocean University in Keelung, Taiwan. The cultured microalgae strain *Chaetoceros muelleri* was supplied from Tungkang Marine Laboratory. Walne's medium was added into culture vials of the microalgae for supplementing its growth nutrient. The microalgae culture environment was maintained at 25-26°C and illuminated with fluorescent lamp of 12000 Lux. Air was pumped into the vial to stir water so that fluorescent light and carbon source can be easily absorbed by microalgae for photosynthesis.

A spectrophotometer was used to determine the light absorption characteristics and the optical density (OD) of the microalgae in order to obtain the correlation with the algal biomass. The crude lipid extracted from microalgae was transesterified to produce fatty acids methyl esters (FAME). Gas-liquid chromatography was used to analyze the composition of the microalgal biodiesel.

III. Results and Discussion

A correlation equation between the dry weight of microalgae biomass and OD value of the culture water was established. Figure 1 shows the biomass of *Chaetoceros muelleri* cultured in DSW reached 292 mg/L, which is significantly larger than 178 mg/L of the microalgae cultured in SSW on the same 11^{th} culture day. This is attributed to the rich nitrate, silicate and phosphate contained in DSW facilitating microalgal growth. The microalgae cultured in DSW also showed higher quantities of both crude lipid and FAME.

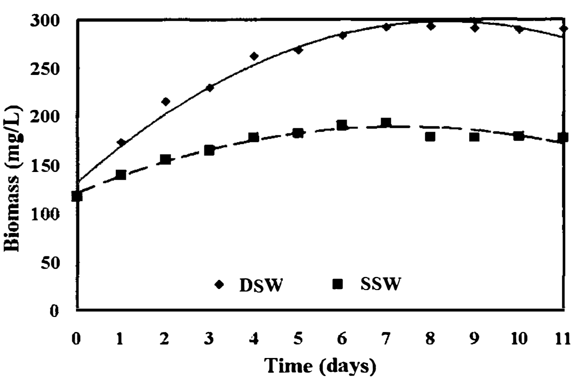

Figure 1. Biomass variation with culture time in DSW and SSW for *Chaetoceros muelleri*

一般講演２
〈海洋･水質関連／生物･水産関連２〉
Session 1: Water Quality, Biology and Fisheries 2

座長　　周　宏農

（国立台湾大学　漁業科学研究所　教授兼所長）

Chairman: Hong-Nong Chou

(Institute of Fisheries Science, National Taiwan University, Professor and Director)

6.DGGE 法による伊豆赤沢海洋深層水中の微生物群集構造解析

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【目的】

海洋は新規有用微生物の宝庫の可能性を秘めているにもかかわらず，これまで微生物の探索源としては海底堆積物や海洋生物が中心であった．従って海水中から分離された微生物，さらには海洋深層水（以下，DSW と記述）中に存在する微生物に関する基礎的知見は乏しいのが現状である．

そこで本研究では，太陽光が殆ど届かない補償深度（水深約 200 m）以深の海水であり，年間を通じて水温が低く，高い清浄性を有し，無機栄養塩が豊富に存在するなどユニークな特徴を有する DSW から，新規微生物，あるいは特異的な生理・生化学的諸性状を有する微生物を分離することに先立ち，まず DSW 中の微生物群集組成を調べることを目的とした．

【方法】

DSW の取水

静岡県伊東市伊豆赤沢にある㈱DHC 海洋深層水研究所の取水施設において，2012 年 1 月から 12 月まで 1 ヶ月ごとに DSW（水深 800 m）を取水した．

微生物 DNA の抽出・精製

取水後，DSW 2 L を孔径 3.0 μm のヌクレオポアフィルターでプレ濾過後，得られた濾液をさらに孔径 0.2 μm のヌクレオポアフィルターを用いて濾過集菌した．それぞれのフィルターから CTAB 法を用いて DNA を抽出・回収した．これに 3 M 酢酸ナトリウム，イソプロピルアルコールを順に加えて精製し，更に 70%エタノールを加えて DNA を精製した後，純水に溶解して -20° C で保存した．なお比較対照として DSW を取水した直上の表面海水を用いて同様の操作を行い，得られた DNA 精製物を-20℃で保存した．

PCR および DGGE 解析

一般細菌は，ユニバーサルプライマー（GC クランプを付与した GC341F，907R）を用いて一般細菌の 16S rRNA 遺伝子を増幅した．乳酸菌は，特異的プライマーを使用し，Nested-PCR を行った．1st PCR では WLAB1 と 1492R を用い，2nd PCR ではテンプレートに 100 倍希釈した 1st PCR 産物，WLAB1 と GC クランプを付与した GCWLAB2 を用いて 16S rRNA 遺伝子を増幅した．また，真菌については，ユニバーサルプライマー（GC クランプを付与した GCNL1，LS2）を用いて，26S rRNA 遺伝子を増幅した．こうして得られた各微生物の PCR 産物を DGGE に供した．

【結果および考察】

一般細菌，乳酸菌および真菌の群集組成を DGGE法で解析した結果，DSW中にも多様な群集組成が存在することが示唆された．そこで，これらの群集組成をMDS解析したところ，各菌種により群集組成が異なることがわかった．次に DGGEゲルから切り出したDNAバンドより微生物の同定を試みたが，一般細菌においては未培養クローンが多く，多数の難培養クローンの存在が示唆された．乳酸菌としては4種が同定されたが，未培養クローンも多かった．一方，真菌と同定されたDNAバンドは9本あったが，近縁種との相同性は85-94%に過ぎないことから，新規菌種の可能性が示唆された．

このようにDSW中の一般細菌，乳酸菌，および真菌の群集組成は多様であり，難培養微生物も多く存在していることが示唆されたが，この点については再現性を含め，今後も継続して調査する必要があると考えている．自然界においても稀有な環境であるDSW中には未知微生物，あるいは特異的な生理・生化学的諸性状を有する微生物の存在が期待できることから，DSWは応用微生物学的見地からも有益な分離源であると考えられる．

6. Microbial community structures of deep seawater in Izu-Akazawa as revealed by DGGE analysis

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Introduction

Although there are prospects for discovering new and useful microorganisms in the ocean, marine sediments and marine organisms were not seriously considered in the search for such microorganisms until now. As a result, limited basic knowledge of microorganisms from seawater and deep seawater (DSW) is available. This research was aimed at describing microbial community structures in DSW before the isolation of new component microorganisms that were previously characterized phenotypically.

Method

Sampling of deep seawater

DSW samples were collected from DHC Corporation's Izu-Akazawa DSW pumping station in Shizuoka Prefecture every month from January to December, 2012.

Extraction and purification of microbial DNA from DSW samples

After 2 L of DSW samples were filtered with a Nuclepore filter (pore size, 3 μm) to separate attached microbes, the filtrate was also treated with a Nuclepore filter (pore size, 0.2 μm) to separate free-living microbes. Bacterial DNA on these filters were extracted and collected by the C-TAB method. The purified DNA was kept at -20℃ after the collected DNA was purified with 3M sodium acetate, isopropyl alcohol and 70% ethanol. In addition, surface seawater samples from Izu-Akazawa were treated similarly as samples for comparison.

PCR and DGGE analysis

The primers GC341F and 907R were used for amplifying universal DNA fragments of the bacterial 16S rRNA gene fragments (rDNA). Nested-PCR was used for lactic acid bacteria. The primers WLAB and 1492R were used in first PCR for amplifying lactic acid bacteria specific-DNA fragments. The amplified DNA was diluted by a factor 100 and used as the template for the second PCR amplification with the primers WLAB1 and GCWLAB2. The primers GCNL1 and LS2 were used for amplifying universal DNA fragments of fungal 26S rDNA. The PCR product from each sample was analyzed by denaturing gradient gel electrophoresis (DGGE).

Result and discussion

From the microbial community structures of bacteria, lactic acid bacteria and fungi analyzed by DGGE, it was suggested that various microbial community structures exist in DSW. The multi-dimensional scaling (MDS) map generated from DGGE profiles showed that community structures are different for every microbe. Samples of individual bands excised from the gel were examined to identify a species. However, it was suggested that there were a lot of uncultured microbes because there were also many unknown bacterial clones. Four dominant bands were identified to lactic acid bacterium, although there were also a lot of uncultured microbes. On the other hand, nine dominant bands were identified as fungus, even though the homogeny with relative species was only 85 to 94%. Therefore, the possibility that they were novel fungi exists.

Thus, there were various microbial community structures of the standard bacteria, lactic acid bacteria and a fungus in DSW samples, and it was suggested that many uncultured microbes also existed. However, it seemed that it is necessary to research further in the future to follow on this result.

It seems that DSW with its peculiar environment is a useful starting point for the isolation of microbes useful in industrial microbiology, because it is expected that there are unique microbes with distinct phenotypic characterization.

7. 以深層海水為培養基可提高本土微藻 *Chlorella sorokiniana* CY1 之生長速率與油脂含量

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[4]財團法人石材暨資源產業研究發展中心水資源組)

一、 前言

全球能源需求的约 80%來自化石燃料, 然而, 廣泛利用化石燃料導致全球氣候變化, 環境污染和健康問題(Chen et al., 2011), 因此, 許多國家都將注意力轉向乾淨, 可再生能源的發展。在眾多的可再生能源中, 生質燃料是最有潛力且符合環保規範 (Chisti, 2007)。微藻生長迅速, 利用 CO_2 固定將太陽能轉化為化學能, 因此, 被視為最有希望應用於製造生質柴油的新興料源之一 (Mata et al., 2010)。

與其他能源作物做比較, 微藻具有較高的油脂生產力, 成為備受關注的生質柴油原料(Chisti, 2007; Haag, 2007)。現今已應用一些策略去提高微藻生長和油脂含量, 這些策略包括培養基的優化（例如不同的碳源成分、維生素、無機鹽和營養物質), 物理參數(如pH值, 溫度和光照強度), 和不同類型的代謝（例如, 自營、異營和混營）(Chojnacka and Noworyta, 2004; Yeh et al., 2012)。其中, 使用不同的能量來源與碳源（無機或有機）一直被視為影響微藻生長和脂質蓄積的關鍵因素之一 (Chen et al., 2011, Chen et al.2010a,b)。

深層海水之定義為深度200公尺以上的海水, 此層海水照不到陽光, 不易受到外在環境影響, 與淺層海水相比, 富含營養源與微量元素, 水質相對穩定與乾淨(Nakasone and Akeda, 1998), 故近年也來被應用於藻類之培養。

二、 實驗方法

藻種來源

本研究使用之藻株為 *Chlorella sorokiniana* CY1, 於台灣中南部水域所篩選到的本土藻種。

藻體培養與分析方法

本研究利用 TL5 日光燈管進行全日光照, 並以 0.2 vvm 之曝氣速率通入 2%之 CO_2 氣體。每日進行藻液取樣, 以 pH meter 測量其酸鹼值, 並以分光光度儀(UV-VIS)於吸收波長 680 nm 下測定其吸光值, 再將之換算成藻體細胞乾重以得藻體濃度。此外, 將上清液以分光光度儀在吸收波長 220 nm 下測定其吸光值, 將值代入檢量線得其培養基中氮源濃度。

三、結果與討論

本研究使用高油脂含量之藻種 *Chlorella sorokiniana* CY1, 以深層海水當作培養基並以插入式 LED 為內部光源, 探討其對該藻株之藻體濃度及油脂產量之影響。結果顯示, 插入式 LED 光源能有效提升油脂產量, 其油脂含量和藻體濃度分別達到 53.8%和 2.6 g/l。接著, 探討不同進料策略對藻體濃度以及油脂產量之影響, 結果顯示利用半批次之進料策略確實能夠有效提升藻體濃度及油脂產量。最後以半批次的操作策略, 探討不同置換比例(25%, 50% 及 75%)對藻體濃度以及油脂含量之影響, 發現當置換比例控制在 50%時, 該藻株具有較高的產油效能, 其藻體濃度以及油脂產量分別達到 2.7 g/l 和 61.5%。分析於微藻內脂肪酸之組成, 主要為棕櫚酸(C16:0)、硬脂酸(C18:0)、油酸(C18:1)、亞油酸(C18:2)和二十碳烯酸(C20:1), 此脂肪酸組成相當適合應用於生質柴油之生產。

7. Enhancing microalgal oil/lipids production from *Chlorella sorokiniana* CY1 using deep-seawater as the cultivation medium

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I. Introduction

Today about 80% of global energy demand is produced from fossil fuels. However, extensive utilization of fossil fuels has led to global climate change, environmental pollution, and health problems (Hallenbeck and Benemann, 2002). Many countries are thus turning their attention to the development of new, clean, and sustainable energy sources. Among the various potential sources of renewable energy, biofuels are of most interest and are expected to play a crucial role in the global energy infrastructure in the future. Consequently, microalgae that can grow rapidly and convert solar energy to chemical energy via CO_2 fixation are now being considered a promising oil source for making biodiesel (Mata et al., 2010).

Microalgal lipids have attracted much attention as future raw materials for biodiesel synthesis due to the potential of attaining much higher lipid productivity over other lipid based energy crops (Chisti, 2007; Haag, 2007). Several strategies have been applied to improve microalgae growth and lipid content. These include optimization of the medium compositions (e.g., type of carbon source, vitamins, salts and nutrients), physical parameters (e.g., pH, temperature and light intensity), and type of metabolism (e.g., phototrophic, heterotrophic, mixotrophic and photoheterotrophic growth) (Chojnacka and Noworyta, 2004; Yeh et al., 2012). In particular, the type of cultivation method, using different energy sources (light or organic) and carbon sources (inorganic or organic), is always recognized as a key factor that significantly influences the growth and lipid accumulation of microalgae (Chen et al., 2011; Chen et al., 2010a; Chen et al., 2010b).

Deep seawater generally refers to seawater from a depth of more than 200 m. Deep-seawater is known to contain some specific trace elements and nutrients that could be beneficial for the growth of microalgae or for stimulating the production of specific components in the microalgae.

II. Materials and Methods

Isolation of microalgae

The microalgal strain *Chlorella sorokiniana* CY1 used in this work was isolated from freshwater located in southern Taiwan.

The microalgae culture

C. sorokiniana CY1 strain was grown under a light intensity of approximately 150 μmol/m^2/s (illuminated by TL5), 2% CO_2. The OD680 values were converted to biomass concentration via proper calibration between OD680 and dry cell weight (i.e., 1.0 OD680 approximately equals 300 mg DCW/l).

III. Results and Discussion

Microalgae are considered as one of the most promising renewable feedstock for use in biofuel production due to their advantages of fast growing, higher carbon dioxide fixation efficiency, and potentially containing high contents of oil/lipids. In this study, the autotrophic growth and oil accumulation of an oil-rich indigenous microalga *Chlorella sorokiniana* CY1 was promoted by applying engineering strategies using deep sewater as the medium. First, the microalga was cultivated using LED as the immersed light source to stimulate the production of microalgal biomass and oil/lipid. The results show that the immersed LED could effectively enhance the oil/lipid production, leading to an oil/lipid content and algae biomass concentration of 53.8% and 2.6 g/l, respectively. Next, the semi-batch operation of PBR was shown to improve the performance of oil/lipid content and microalgae biomass concentration over the performance of batch and CSTR cultures under similar operation conditions. Finally, different replacement ratios (25%, 50% and 75%) in semi-batch cultivation using deep seawater were also investigated. These results indicated that a replacement ratio of 50% could markedly enhance the oil/lipid content and microalgae biomass concentration to 61.5% and 2.7 g/l, respectively. Analysis of fatty acid composition showed that the main fatty acids in the microalgal lipid were palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and eicosenoic acid (C20:1). This lipid quality is suitable for biodiesel production. The knowledge obtained from this study is useful for assessing the feasibility of using deep-sea water to cultivate microalgae for biodiesel production.

8. 海洋深層水中の微生物の群集組成解析と有用微生物の分離

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1 目的

低温、高水圧などの特異環境から取水された海洋深層水（DSW）は、応用微生物学的見地からも様々な利用が期待されるが、微生物に関する基礎的知見は乏しいのが現状である。そこで、本研究ではDSWと表面海水（SSW）中に存在する微生物群集を変性剤濃度勾配ゲル電気泳動法（DGGE）によって調べるとともに、伊豆赤沢のDSWから実際に乳酸菌や酵母を分離してその諸性状を調べることを目的とした。

2 方法

北海道羅臼、岩内、新潟県佐渡島、伊豆大島、伊豆赤沢、駿河湾、高知室戸、沖縄久米島の8箇所の取水設備から2009年3月から2010年1月までDSWとSSWを入手した。それぞれの試料を孔径3.0μmのフィルターで濾過した後、濾液をさらに0.2μmのフィルターで濾過して現場微生物を集菌した。このフィルターから微生物DNAを抽出して、一般細菌のユニバーサルプライマーを用いてPCR増幅した。これをDGGEに供し、得られた結果を解析した。

3 結果および考察

PCR-DGGE法により全国8箇所の取水設備のSSWとDSW中の微生物の群集組成を解析した。その結果、図1に示すように海域ごとに特有の群集組成が見られた。一方伊豆赤沢では図2に見られるように、SSWでは群集組成に季節変化が見られたが、DSWではSSWと比較して変化が少なく、年間を通じて安定しており、さらにSSWには見られない群集の存在が確認された。そこで、この赤沢のDSWの懸濁物の除去に用いられるバッグフィルター（約3万トンのDSWを濾過済み）を入手し、フィルター底部に存在する懸濁物から各種分離培地を用いて乳酸菌と酵母の分離を試みた。その結果、38株の乳酸菌を分離し、同定した結果、35株は*Lactobacillus plantarum*、3株は*Pediococcus pentosaceus*と同定された。これらは陸上由来の植物性乳酸菌として有名であるが、我々の分離株は増殖温度の上限が標準株よりも低いことから、深海環境に適応していると推定された。このうち、*L. plantarum* BF-13株と命名された株がB16メラノーマ細胞の生産するチロシナーゼに対する阻害効果ならびにコラーゲン合成促進作用が見られたため、現在これらの有効物質を培養液上清から単離精製しているところである。また、80株の酵母を分離し、同定した結果、16属23種であることが明らかとなった。このうち、*Saccharomyces cerevisiae*と同定された3株について諸性状を調べた結果、これらの株は標準株と比較して、増殖温度の下限が低く、耐圧性が高く、NaCl耐性も高いことから、深海環境に適応していることが判明した。*Saccharomyces cerevisiae* No. 51株と命名された株は、抗酸化作用が確認された。現在、この活性物質を分離しているところである。

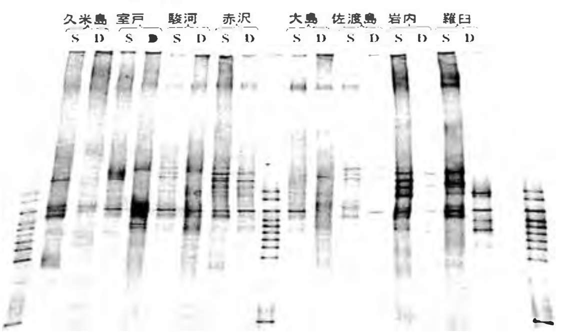

表1 DGGEによる全国8箇所の取水設備の表面海水（S）および海洋深層水（D）の微生物群集組成解析結果

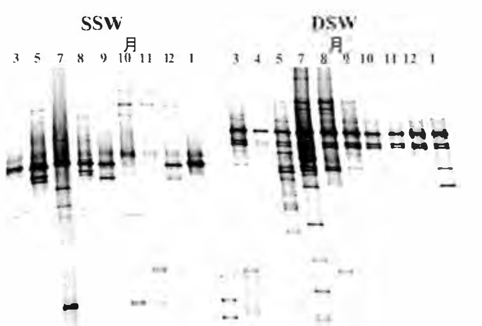

表2 伊豆赤沢のSSWおよびDSW中の微生物群集の季節変化(2009年3月から2010年1月)

8. Analysis of Microbial Community Structure and Isolation of Beneficial Microorganisms from Deep-sea Water

○Chiaki Imada[1], Katsuhisa Yamada[2], Takeshi Kobayashi[1], Takeshi Terahara[1]
([1]Tokyo University of Marine Science and Technology,[2]DHC Corporation)

1. Introduction

Deep-sea water (DSW) collected from extreme environments with low water temperature and high hydrostatic pressure is a promising resource from the applied microbiological point of view. However, research regarding microorganisms living in DSW is still very limited. Therefore, in the present study, microbial population analysis of DSW and surface seawater (SSW) was performed by denaturing gradient gel electrophoresis (DGGE). In addition, useful microorganisms such as lactic acid bacteria and yeasts were isolated from Izu-Akazawa DSW and characterized.

2. Materials and methods

DSW and SSW were collected from 8 pumping stations in Japan such as Hokkaido Rausu, Iwanai, Sado, Izu-Oshima, Izu-Akazawa, Suruga, Kochi-Muroto, Okinawa-Kumejima from March 2009 to January 2010. Collected DSW and SSW were filtered using 3.0 m pore size filter. Then filtrates were further filtered using 0.2 m pore size filter. The microbial DNA was extracted from the suspended solid trapped by the 0.2 m pore size filter and amplified by PCR using universal bacterial primers. The PCR products were analyzed by DGGE.

3. Results and Discussion

Fig. 1 shows the result of the analysis of microbial population of DSW and SSW in 8 pumping stations in Japan. The structures of DSW were different from those of SSW in all the stations. Horizontal variations of the structures from northern (Hokkaido) to southern part (Okinawa) of Japan were also observed. Fig. 2 shows the result of the analysis of microbial population of DSW and SSW in Izu-Akazawa. The actinomycete community structures in SSW varied seasonally, whereas those in DSW were stable throughout the year. Notably, a unique population was observed in DSW.

Various useful microorganisms such as lactic acid bacteria and yeasts were isolated from DSW in Izu-Akazawa. The bag type filter, which was used for the removal of the suspended solid from approximately 30,000 tons of DSW, was used for the isolation of DSW microorganisms. Thirty-eight lactic acid bacterial strains were isolated by MRS medium containing DSW and identified. Thirty-five strains were identified as *Lactobacillus plantarum* whereas three strains were identified as *Pediococcus pentosaceus* by 16S rDNA analysis. These strains are well-known as terrestrial plant lactic acid bacteria. However, our isolates failed to grow in higher temperature like the type strain, suggesting that they have adapted to deep sea environment although they were of terrestrial origin. Culture supernatant of one of our isolates named as *L. plantarum* strain BF-13 had inhibitory activity against tyrosinase from B16 melanoma cells. The activity is higher than those of kojic acid and arbutin which are often used in skin whiteners in the cosmetic industry. It also stimulated collagen synthesis.

Eighty yeast strains were also isolated by YPD medium containing DSW from the filter. Sixteen genera of 23 species were identified by 23 rDNA analysis. Among them, three *Saccharomyces cerevisiae* strains were selected and used for further study. They grew in the low temperature, and had tolerance of hydrostatic pressure, and high NaCl tolerance, suggesting that they have also adapted to the deep sea environment. One strain named as strain No. 51, had anti-oxidative activity. The isolation of active substances from the strains is now underway.

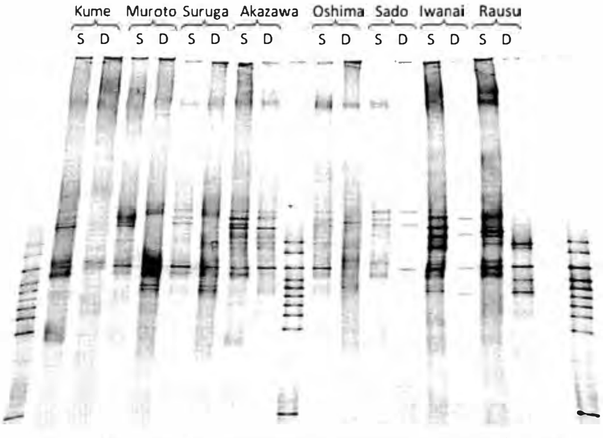

Fig 1. The bacterial community structures of SSW(S) and DSW(D) collected from 8 pumping stations by DGGE

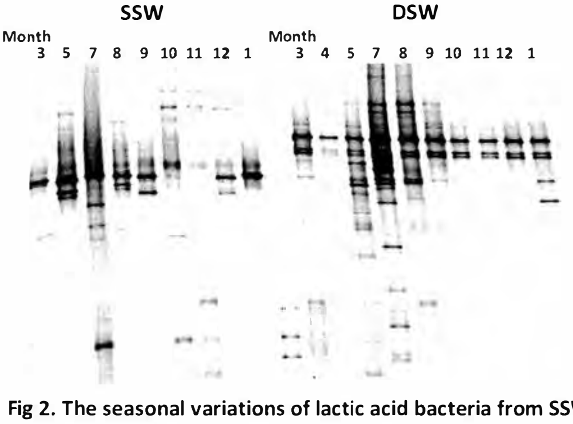

Fig 2. The seasonal variations of lactic acid bacteria from SSW and DSW at Izu-Akazawa by DGGE (March, 2009~Jan. 2010)

9. 探討深層海水對毒源藻類生長及毒素釋放之影響

°林怡汝、陳睿哲、簡世勇、黃秉益(財團法人石材暨資源產業研究發展中心)、
周宏農(國立台灣大學漁業科學研究所)

1.前言

深層海水為台灣東部的一項特色水資源，具有低溫、富營養鹽、潔淨、性質穩定等特性。但由於深層海水取水不易，為了充分利用該資源的特性，台灣深層海水產業已朝多目標及多段式利用的發展，其中藻類生技產品之應用為企業界關注的焦點之一，期望達到高值化、高階應用等多重功效。眾多藻類生技產品中，特定微細藻類所產出的藻類毒素標準品由於具醫藥應用之潛力，逐漸受到重視，本研究針對深層海水對提昇銅鏽微囊藻 *Microcystis aeruginosa* 及利瑪原甲藻 *Prorocentrum lima* 之藻細胞密度及增加毒素含量的培養技術進行初步的探討。

2.實驗方法

利瑪原甲藻及銅鏽微囊藻之藻株來自台灣大學漁業科學研究所周宏農教授所建立之藻類種原庫，分別以 F/2 及 BG-11 培養基培養。

- **反應器效能試驗**

比較鼓泡式及氣舉式光反應器對藻細胞密度的影響。

- **深層海水效益測試**

利瑪原甲藻以表層海水批次追加 F/2 培養基的模式培養至生長穩定期，實驗組以深層海水，對照組則以表層海水為培養液進行培養，在一定時間後比較兩組間藻細胞密度及藻毒產生情形。

銅鏽微囊藻則以 RO 水批次追加 BG-11 的模式培養至生長穩定期，再以深層海水濃縮液(硬度 0-3000 ppm)置換 BG-11 培養基或添加深層海水原水（0-1%）觀察深層海水對藻細胞密度及藻毒產生的影響。

3.結果與討論

實驗結果顯示氣舉式光反應器因循環攪拌的關係質量傳遞的效率好，所以不論利瑪原甲藻或微囊藻增殖速度皆明顯比以鼓泡式光反應器培養快。比較深層海水與表層海水對利瑪原甲藻生長的影響發現深層海水可促進利瑪原甲藻的增殖，在添加一次 F/2 營養鹽的環境中使用深層海水進行培養的結果，藻細胞密度達表層海水組的 1.6 倍。以表層海水進行利瑪原甲藻高密度培養時，藻細胞出現停滯增殖的現象，而使用深層海水則能讓藻細胞持續增殖，且維持一定的單位細胞的淨產毒率。另一方面，添加深層海水濃縮液（硬度 0-3000 ppm）及原水（鹽度 0-1%）皆不影響微囊藻的增殖及單位細胞的淨產毒率。

由此結果顯示，以氣舉式光反應器培養利瑪原甲藻或微囊藻有助於藻細胞生長。深層海水有利於促進利瑪原甲藻增殖，且可維持一定的淨產毒率。但是，對屬於淡水藻的微囊藻添加深層海水對藻細胞增殖及單位細胞的淨產毒率則沒有顯著的影響。

9. Effects of deep seawater on algal growth and algal toxins release

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([1] Stone & Resource Industry R&D Center, Hualien, Taiwan,
[2] Institute of Fisheries Science, National Taiwan University, Taipei, Taiwan)

I. Introduction

Deep seawater (DSW) has a relatively constant temperature and abundant nutrients, such as nitrate and phosphate, which are suitable for use in algal culture. DSW possesses clean properties, posing no risk of toxic pollutants; it can also be used as a microalgal fertilizer. Thus, the properties of DSW facilitate farming economically valuable algae. *Microcystis aeruginosa* is a principal bloom-forming cyanobacteria which is present in a wide range of freshwater ecosystems. *M. aeruginosa* produces microcystin that can induce hepatotoxicity, harming humans and animals. Harmful algal blooms (HABs) severely affect the economy and public health, because phycotoxins travel along the food chain, contaminating edible shellfish and causing toxic incidents. *Prorocentrum lima* plays a major role in HABs, producing okadaic acid, as well as inducing diarrhetic shellfish poisoning in humans.

This study involved using DSW, surface seawater, and brine made from DSW (nigari) to cultivate *P. lima* and *M. aeruginosa*. We observed the growth rates and concentration levels of algal toxins and investigated the feasibility of using DSW to cultivate *P. lima* and *M. aeruginosa*.

II. Materials and Methods

P. lima and *M. aeruginosa* were obtained from the Institute of Fisheries Science, National Taiwan University and cultivated in F/2 and BG-11 media. The cell densities of the cultures were spectrophotometrically monitored at 680 nm (OD680). The algal toxins were analyzed using HPLC.

Reactor ability test:

We compared how using bubbling and an air-lift photo-bioreactor affected the algal cell density.

Deep seawater efficiency test:

P. lima was cultivated to a stable phase in F/2 medium. Subsequently, the effects of DSW and surface seawater were compared by measuring algal cell density and algal toxin production levels. *M. aeruginosa* was cultivated to a stable phase, subsequently displaced in BG-11 medium, and combined with nigari (hardness 0–3000 ppm) or DSW (0%–1%). We examined how DSW affected algal cell density and algal toxin production levels by using spectrophotometric monitoring and HPLC.

III. Results and Discussion

An air-lift photo-bioreactor increased the growth rates of both *P. lima* and *M. aeruginosa*. The *P. lima* growth rate was enhanced to approximately 60% in DSW; however, using DSW did not influence the release of algal toxins. Neither nigari nor DSW affected the growth rates or algal toxin release levels of *M. aeruginosa*.

10. 日本国における沿岸漁業の課題解決のための海洋深層水の利用

山内繁樹、筒井浩之（株式会社エコニクス）

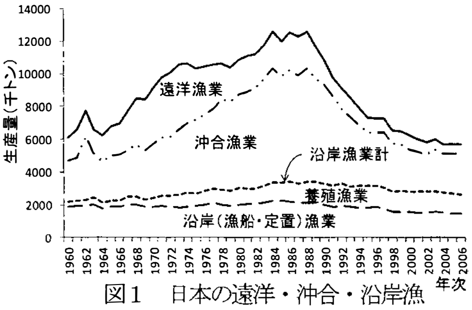


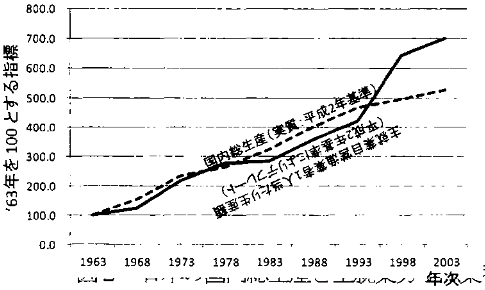
図１　日本の遠洋・沖合・沿岸漁業の生産量の推移

１　はじめにかえて（日本国沿岸漁業の現状）

日本の沿岸漁業生産は，1960 年以降変動は認められるものの大きな変化はないことが知られている。しかし，この間の沿岸漁業者の減少は著しく主就業者数（65 歳未満，ただし 1968 年以前 60 歳未満）は 2008 年では 1963 年の 21％となっている。この間，老齢者就業者数（65 歳以上，ただし 1968 年以前は 60 歳以上）は 90％となっているが大きな変化とは言えない。生産一定と主就業者数の低下は１人あたりの生産性の増加を示すものであり生産性の増加は日本の経済上昇率とほぼ合致している。この事実は日本の沿岸漁業地域が所定の生産量に対し経済成長とともに生産性を上げ労働力を減少させていることを示す。すなわち，地域の衰退は生産性の向上と労働力に見合った生産量の増加が得られないところにある。この基本的構造を打開する深層水を含む海水利用の方向・手法について提案する。

２　地域課題の解決と海洋深層水

地域沿岸漁業の停滞は地域海面積に由来する資源が限定（一定）されるからである。地域維持には国内の経済成長に応じた生産の持続的拡大が必要であり，持続的拡大には拡大供給可能な資源と資源を経済的に利用する技術が必要である。沿岸域での適切な生産対象は水産生物である。これを生産するための基本的資源は海水と生産対象となる種の種苗である。海水は深層水を含め巨大な循環を持つ再生資源である，また，沿岸種であるアワビ，ウニの種苗は企業的な拡大生産が可能となっている。



１人あたりの沿岸漁業生産額
（デフレータにより修正）

３　深層水利用と飼育収容技術

拡大供給が可能な資源を経済価値化するためには収支が見合う製造技術が必要となる。深層水の供給にはコストが生じる。従って，単位水量に対する生産を可能な限り拡大することが技術の核心となる。生物生産では種苗を飼育し増大させ，単位（時間，密度，流量）あたり生産量Gは個体重量ｇ× 個数ｎで表すことができる。 個体重量ｇは生物量であり深層水と表層水の混合による飼育水温の調整などによる促進が考えられるが飛躍的に向上させることは困難である。個数ｎは物理量であり，その拡大は収容技術の開発により比較的容易と考えている。本報告では２例の収容技術を紹介する。

１）流れによる収容密度の増加

飼育水に流速を与えることにより飼育密度を向上させることが知られている（浮他　編著　1995）。図３は流れを利用する流下堰水槽（特許第 3493357 号）による飼育収容の例を示している。この事例では約２.５ｃｍの水位でアワビの飼育を可能としている（干川・山内他　2012）。

２）空間利用による収容密度の増加

ウニの飼育は着生面を必要とする。通常，付着面は平面であるが，図４では垂直に付着させ単位面積・空間（水量）あたりの飼育数量を向上させる例を示している。この事例では 500 個／m^2 の収容を可能としている（高橋他　1994）。

４　おわりに

養殖により沿岸漁業生産を持続的に向上させるためには「従来の生物主体の技術では限界にきている」と考えている（佐野　1988）。今後の沿岸漁業の持続的発展には物理工学的観点を導入した深層水の利用が有効であるものと考えられる今後の技術開発を期待したい。

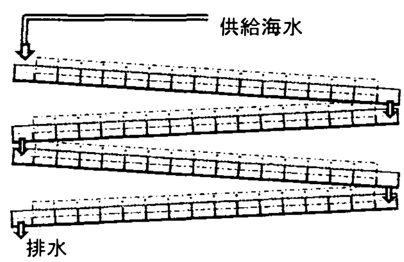


図３　流下堰による流れを利用したアワビ飼育収容の概念図

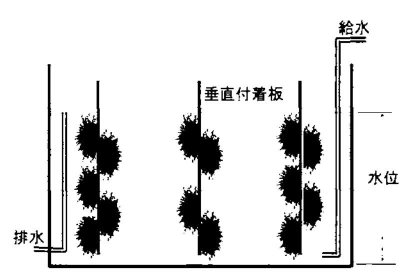


図４　垂直付着板を利用したウニ飼育収容の概念図

10. Utilization of deep-ocean water for the solution of coastal fishery problems in Japan

°Shigeki YAMAUCHI, Hiroyuki TSUTSUI (ECONIXE Co. Ltd.)

1 Introduction

There has been no substantial change in the coastal fishing production of Japan since 1960. However, during this time the number of coastal fisherman has decreased remarkably. The number of primary workers (under age 65 or under age in pre 1968 date) in 2008 was only 21% of the 1963 number. However, there has been no substantial change in the number of elderly fishermen .Their number in 2008 was 90% of the 1963 number. Constant levels of production combined with a decrease in the number of primary workers suggest an increase in per capita productivity. Further, this increase in productivity almost exactly follows the rate of economic growth in Japan. This fact shows that Japan's coastal fishing regions are experiencing a decrease in manpower due to economic growth and increased productivity in contrast with constant production levels. That is, the decline of the region is happening because there has been no increase in production to match manpower and improvements in productivity. To solve the problems caused by this basic structure, we propose directions and techniques for the use of seawater, including deep ocean water.

2 Solutions for regional issues and the use of seawater

The reason why regional coastal fishing has stagnated is that resources that depend on sea surface area are limited. Maintenance of the region's continued expansion of production in line with domestic economic growth is needed. For continued expansion, resources that can expand their supply, and technology that can economically exploit those resources are both necessary. The appropriate object of production for coastal regions is marine products. Seawater, including deep ocean water, is a renewable resource with a huge circulation. Moreover, eggs and hatchlings of abalone and sea urchin, (which live in coastal waters) could possibly support expanded corporate production.

3 Technology for breeding capacity and the utilization of deep seawater

Cost-effective technology is needed in order to economize resources with potential for supply expansion. There is a cost to supply deep seawater. Thus, the technology should expand production per unit of water as much as possible. In the production of living things, it is necessary to increase breeding. Production for each on it (time, density, and flow quantity) G can be shown as the individual g times the number of individuals n. The individual weight is difficult to greatly increase, though some improvements might be made through the mixing of deep seawater and surface seawater. The number of individuals is a physical value, and its expansion should be comparatively easy through the development of capacity techniques. We will introduce two examples of techniques to increase capacity in this report.

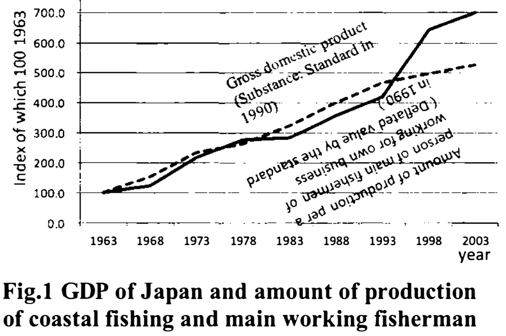


Fig.1 GDP of Japan and amount of production of coastal fishing and main working fisherman (Correct it by the deflator).

1) Increase in containment density through flow

It is known that increasing flow velocity improves breeding density. (Uki *et. al.*). The concept of breeding that uses flow is shown in figure 3. The water tank used is a flowing water tank (patent No.3493357). It is possible to breed abalone in about 2.5 cm of water in this system. (Hoshikawa and Yamauchi *et al.* 2012)

2) Increase in containment density by space use

Breeding of the sea urchin needs the adsorption side. The adsorption side of the sea urchin is usually a plane. However, the adhesion to the vertical in Figure 4 increases the amount of breeding for the unit area and for each space (volume of water). The accommodation of 500 sea urchins/m^2 is possible in this case (Takahashi *et al.* 1994).

4 Conclusion

. Breeding of sea urchins requires adsorption space. The space for adsorption for sea urchins is usually a horizontal plane. However, adhesion to a vertical plane as in figure 4 increases the amount of breeding per unit area and for each apace (volume of water). 500 sea urchins per square meter can be accommodated in this system (Takahashi *et al.*1994).

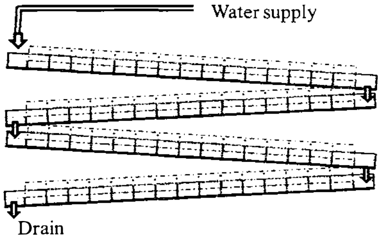


Fig.2 Conceptual diagram of horizontal breeding set-up using flow

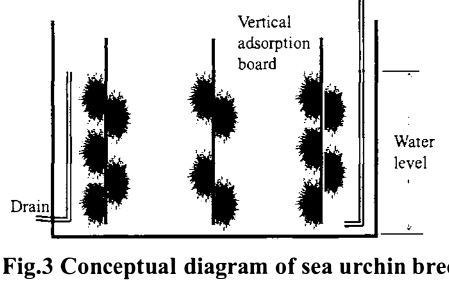


Fig.3 Conceptual diagram of sea urchin breeding set-up using vertical adhesion board

11. 利用深層海水培育潔淨餌料生物之可能性

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1. 前言：

所謂的深層海水是指有光層以下的海水，相較於表層海水，深層海水中微生物與病原菌等生物性及化學性汙染物幾乎不存在，潔淨度高且營養鹽豐富。因此，深層海水性被期待可運用於生產乾淨、優質的水產餌料生物。

本研究使用深層海水進行動物性 (輪蟲、豐年蝦和劍水蚤等) 與植物性餌料(周氏扁藻、牟氏角毛藻、等鞭金藻及海洋擬球藻)的培育測試。期望能藉由深層海水的導入，建立高營養、高經濟與無特定病源菌感染的水產種苗用餌料生物之生產技術，以降低病毒感染的危害，有助於改善現今養殖的窘況。

2. 實驗方法：

挑選兩種水產養殖常用動、植物性餌料生物，植物性餌料生物是擬球藻(*Nannochloropsis oculata*) 和等鞭金藻(*Isochrysis galbana*)，使用PG培養基培育。培育條件為光照10,000Lux±20%，光照週期10_{hr}:14_{hr}(明：暗)，鹽度34‰，pH7.8±20%，溶氧(DO)6.5mg/L±20%，溫度25±2℃。

動物性餌料生物使用輪蟲(Rotifer)和豐年蝦(Artemia)，並以深層海水培育之擬球藻作為其餌料，培育條件為光照7,000Lux±20%，光照週期8_{hr}:16_{hr}(明：暗)，鹽度34‰，pH7.8±20%，溶氧(DO) 6mg/L±20%，溫度25±2℃。動、植物性餌料生物分別都利用表層海水及深層海水培育並收集，持續擴培至10世代，收集後抽取DNA。

本研究以水產養殖常見的兩種病毒，石斑虹彩病毒(GIV)與神經壞死病毒(NNV)的基因序列設計引子(primer)為標的。抽取動、植物餌料生物的DNA為模板進行聚合酶鏈鎖反應(PCR)後，將反應後的DNA片段進行定序與定量分析。病毒序列利用NCBI資料庫進行比對。

3. 結果與討論

實驗結果，利用深層海水培育動物性餌料生物的孵化率為95％、活存率達90%，與利用表層海水培育之孵化率為90％、活存率達80%相較之下表現較好。另一方面，以深層海水培養植物性餌料生物的密度達$3.6x10^6$以上，同表層海水則為$2.8x10^6$以上，證明了深層海水在水產餌料生物之生產過程具有提升其生長速率的可能性。

在培育一定時間後以分子生物技術進行病毒檢測之結果，使用表層海水培育的動、植物餌料，在每一個世代皆能偵測到病毒的存在。而使用深層海水培育的餌料，則皆未檢測出任何一種病毒的反應。以上結果顯示潔淨的深層海水在生產優質潔淨餌料生物應用上具有相當大的潛力，如可成功擴大其規模，必可協助養殖產業之發展。

11. Prospects for producing clean food organisms for aquaculture using deep sea-water

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I. Introduction

Deep sea-water (DSW) refers to seawater below the euphotic zone. Compared to surface seawater (SSW), DSW has almost no biological or chemical contaminants such as microorganisms, pathogens and persistent organic pollutants (POPs). In addition, DSW is extremely clean and nutrient rich. Consequently, DSW is ideal for producing clean and high quality food organisms for the aquaculture industry.

This study used DSW to conduct food organisms of zooplankton (*Rotifera*, brine shrimp, and *Acanthocyclops*) and phytoplankton (*Tetraselmis chui*, *Chaetoceros muelleri*, *Isochrysis galbana*, and *Nannochloropsis oculata*) cultivation tests. With the introduction of DSW, the researchers aimed to establish a technique for producing highly nutritional and economical food organisms used for aquaculture, without infection from specific pathogens. This reduces the risk of viral infection and is beneficial for resolving current aquaculture difficulties in Taiwan.

II. Experimental Method

For this study, 2 species of each zooplankton and phytoplankton group commonly used in aquaculture in Taiwan were selected. Phytoplankton of *Nannochloropsis oculata* and *Isochrysis galbana* were selected and cultured. PG medium which is modified from recipes of Provasoli (1957), and Guillard and Ryther (1962) (Chen, 2000). The culture conditions employed were as follows: light intensity 10,000 Lux ± 20%, photoperiod 10h: 14h (light: dark), salinity 34‰, pH 7.8 ± 20%, dissolved oxygen (DO) 6.5 mg/L ± 20%, and temperature 25 ± 2 °C.

The zooplankton selected for this study were *Rotifera* and brine shrimp (*Artemia*), and DSW-cultured *N. oculata* were used as their food. The culture conditions adopted were as follows: light intensity 7,000 Lux ± 20%, photoperiod 8h: 16h (light: dark), salinity 34‰, pH 7.8 ± 20%, DO 6 mg/L ± 20%, and temperature 25 ± 2 °C. Both the zooplankton and phytoplankton were cultured with SSW and DSW to the 10^{th} generation and then harvested. After harvest, their DNA was extracted.

This study used gene sequences from 2 viruses commonly found in aquaculture farms in Taiwan, namely, the grouper iridovirus (GIV) and nervous necrosis virus (NNV), to design the primer as a target. The zooplankton and phytoplankton DNA were extracted as polymerase chain reaction templates. After the reaction, DNA segments were sequenced and quantitatively analyzed. Viral sequences were compared using the National Center for Biotechnology Information database.

III. Results and Discussion

The results showed that cultivation in DSW yielded a zooplankton with a hatching rate of 95% and a survival rate of 90%. By comparison, cultivation in SSW yielded a hatching rate of 90% and survival rate of 80%. In addition, phytoplankton cultured in DSW possessed a cell density exceeding 3.6 x 10^6cells/ml, whereas the density achieved in SSW reached only 2.8 x 10^6cells/ml. This proved that using DSW for phytoplankton culture can improve their growth yield.

After culture for a defined time period, molecular biology techniques were employed to conduct viral tests. Viruses were detected in every generation of zooplankton and phytoplankton cultured in SSW. However, no viral response was detected in each species cultivated with DSW. These results highlight the substantial potential of using clean DSW to produce high-quality clean food organisms for aquaculture. Successful expansion of the process scale can benefit the development of the aquaculture industry.

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12. 探討深層海水對樟芝菌生長及離子吸收之影響

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1. 前言：

樟芝 (*Antrodia camphorata*) 具有抗癌、保肝、降血壓、抗發炎、抗氧化等功效，功效成分包含三萜類 (triterpenoids)、腺苷 (adenosine)、多醣 (polysaccharide) 與 β-1,3-葡聚醣 (β-1,3-glucan) 等。深層海水 (Deep sea water, DSW) 是位於海平面 200 公尺以下，含有豐富的礦物質與微量元素，可能做為關鍵酵素的輔因子，促進微生物生長或增加代謝產物。前人研究證實 DSW 可促進液態發酵樟芝菌絲體生成，提高三萜類與多醣含量。但過去研究未探討 DSW 對樟芝固態發酵與離子吸收之影響，因此本研究以不同濃度 DSW 為水份來源，進行液態及固態發酵，探討 DSW 對樟芝生長及離子吸收之影響。

2. 材料方法：

本研究之樟芝菌株 *Antrodia camphorata* BCRC 35396 為試驗菌株。DSW 濃縮液 (含 43400 mg/L Mg^{2+}) 購自台灣海洋深層水股份有限公司 (花蓮，台灣)，本研究將上述濃縮液稀釋至 Mg^{2+} 濃度為 20.65 mg/L 做為 1 倍 DSW 之基準。依據上述方法將 DSW 濃縮液稀釋為 5、10 與 15 倍 DSW，分別含 103.25、206.5 與 309.75 mg/L Mg^{2+}，並作為固態與液態發酵主要水分。種菌培養於 28°C、100 rpm 振盪培養 7 天，液態發酵於 1.5 L PDB 培養基中接種 10% 種菌，以 28°C、通氣量 1 v/v/m 培養 18 天，固態發酵分為糙米、糙燕麥、紅薏仁與玉米梗 4 種基質，30 g 基質中接種 10% 種菌，於 28°C 培養。腺苷與 β-1,3-葡聚醣為樟芝功效成份，腺苷以 HPLC 分析，β-1,3-葡聚醣以 aniline blue 分析，並以 xylidyl blue 與 o-cresolphthalein 分別分析發酵液中 Mg^{2+} 與 Ca^{2+} 離子濃度以計算離子吸收量。

3. 結果與討論：

固態發酵中糙燕麥菌絲生長快於其他基質，而 15 倍濃度之 DSW 會進一步促進糙燕麥樟芝菌絲生長。於液態發酵結果顯示，DSW 可促進菌體生長，提高功效成份腺苷與胞內 β-1,3-葡聚醣含量。比較未發酵與發酵後 DSW 培養基之 Mg^{2+} 與 Ca^{2+}離子濃度，Mg^{2+} 與 Ca^{2+} 離子隨著 DSW 濃度上升吸收量隨之上升，然而 DSW 濃度提高至 15 倍時 Ca^{2+} 離子吸收量趨緩。表明樟芝生長及功效成份含量是受到被吸收之離子影響，此外 DSW 中被吸收之離子轉換為有機型態後，可能有益於離子被人體吸收。

12. Effects of deep sea water on the growth and ion absorption of *Antrodia camphorata*

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1. Introduction:

Antrodia camphorata (AC) possesses anticancer, anti-hepatotoxic, anti- hypertensive, anti-inflammatory, and anti- oxidative effects due to triterpenoids, adenosine, polysaccharides, and β-1,3- glucan. Deep sea water (DSW) located 200 m or more below sea level contains rich inorganic nutrients and minerals which can be used as cofactors for key enzymes to promote microbial growth or increase the biosynthesis of metabolites. DSW was proven to increase AC biomass and functional components production of triterpenoids and polysaccharide in submerged culture in our previous study. The ions bio-absorption of AC from DSW in submerged culture and the effect of DSW on the growth of AC in solid culture are unclear. This study investigated the effect of DSW on the growth and ion bio-absorption of AC.

2. Materials and Methods:

Antrodia camphorata BCRC 35396 was used as the fermentation strain. Concentrated DSW (including 43400 mg/L Mg^{2+}) was purchased from Taiwan Yes Company (Hualien, Taiwan). In this study, the concentrated DSW was diluted to the solution including 20.65 mg/L Mg^{2+} and which was defined as 1-fold DSW. According to the above method, the concentrated DSW was diluted to 5, 10, and 15-fold DSW, including 103.25, 206.50, and 309.75 mg/L Mg^{2+}, respectively, and further used as the culture water in solid culture and submerged culture. The seed culture was incubated at 28°C with 100 rpm for 7 d. After that, 10% seed culture broth was transferred to 1.5 L PDB medium and incubated at 28°C with 1 v/v/m aeration for 18 d. In solid culture, 10% seed culture broth was transferred to 30 g solid substrate (brown rice, oat, red pearl barley, and corn cob) and incubated at 28°C. Adenosine and β-1,3-glucan were measured by HPLC and aniline blue method, respectively. Mg^{2+} and Ca^{2+} concentrations in unfermented and fermented medium were detected by xylidyl blue and o-cresolphthalein, respectively, and calculated the bio-absorption of ions.

3. Results and Discussion:

In the solid culture, AC mycelium growth in red pearl barley was faster than that in the other substrates, and 15-fold DSW further stimulated the growth of AC mycelium in red pearl barley. In the submerged culture, DSW promoted AC mycelium growth and increased the adenosine and intracellular β-1,3-glucan contents. Comparing Mg^{2+} and Ca^{2+} concentrations between the unfermented and the fermented DSW media, Mg^{2+} and Ca^{2+} bio-absorption increased with increasing DSW concentration. However, when the DSW concentration reached 15-fold, the Ca^{2+} bio-absorption did not increase, indicating that the growth and functional components levels of AC were possibly affected by the absorbed ion. Additionally, the absorbed DSW ions were transformed to organic ions, which could be beneficial for ion absorption in human.

13. 伊豆赤沢海洋深層水から分離した酵母の酸化ストレス耐性に関する研究

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1 目的

海洋深層水は低温性，清浄性などの特徴から，生菌数が非常に少ないことが知られている．我々は分子生物学的手法を用いた研究により伊豆赤沢海洋深層水(以下,DSW)中には生物量としては少ないものの，微生物群集構造として捉えた場合，直上の表面海水中とは顕著に相違することから，DSWが有用微生物の有力な分離源として有望であることを明らかにしてきた．

先の学会[1)]で報告したとおり，DSW中の酵母に着目して，産業上特に有用な *Saccharomyces cerevisiae* 3株を分離し，これらの諸性状を明らかにした．なおDSWから分離した *S. cerevisiae*（以下，分離株）は，陸上由来の同種（以下，基準株）と比較して耐塩性，増殖温度，経時寿命，酸化ストレス耐性などの性質が異なることを既に明らかにしている。そこで，本研究では酸化ストレス耐性を中心に更なる検討を行ったので報告する．

2 方法

分離株と基準株を，精製水（以下，DW）およびDSWで調製したYPD培地に植菌し，これらの増殖が定常期に達するまで（48時間）培養した．培養後，菌体を回収し，滅菌DWで洗浄後，各濃度に調整した過酸化水素水を添加して酸化ストレス負荷を行った（30℃，1時間）．その後，過酸化水素水を除去するために各菌体を滅菌DWで洗浄し，滅菌生理食塩水に懸濁した．これらの懸濁液10　LをYPD寒天培地に接種した後，27℃で培養を行った．培養後，YPD寒天培地上の酵母の増殖性から，酸化ストレス耐性を比較した．

3 結果および考察

DW培地で培養した場合，分離株は2,240mMの過酸化水素処理でも増殖が見られたことから，基準株（280mMの耐性）より顕著に高い酸化ストレス耐性を示した．一方，DSW培地で培養した場合，基準株の酸化ストレス耐性が280mMから2,240mMへと，飛躍的に向上した（Table）．このことからDSWは *S. cerevisiae* の酸化ストレス耐性の向上に何らかの影響を及ぼしていることが示唆された．

今後 *S. cerevisiae* の酸化ストレス耐性を向上させるDSWの要因について詳細に調べる為に，表面海水や人工海水を用いた培養試験を行う予定である．なお *S. cerevisiae* について，人為的な遺伝子操作により経時寿命を延長させると酸化ストレス耐性も向上することが報告されていることから[2)]，DSWが *S. cerevisiae* の経時寿命に及ぼす影響についても検討したいと考えている．さらに，酵母と同じ真核生物であるヒトの細胞の酸化ストレス耐性に及ぼすDSWの影響についても検討を行う予定である．

Table Influence of various concentrations of hydrogen peroxide on the growth of *S. cerevisiae* cultured in DW or DSW.

Medium	*S. cerevisiae*	Concentration of hydrogen peroxide (mM) 0	280	560	1,120	1,680	2,240
DW	Isolated strain	+	+	+	+	+	+
	Type strain	+	±	−	−	−	−
DSW	Isolated strain	+	+	+	+	+	+
	Type strain	+	+	+	+	±	±

+:Good growth,　±:Poor growth,　−:No growth

(1)海洋深層水研究, 13 (2), pp.98, 2012.
(2)*Biosci. Biotechnol. Biochem.*, 73(12), 2787-2789, 2009.

13. Investigation of the oxidant stress tolerance of the yeast isolated from deep seawater in Izu-Akazawa

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Introduction

It is known that there are few microbes from deep seawater (DSW) in Izu-Akazawa because of special environmental features such as low temperature and cleanliness. We showed that DSW is an important source for the isolation of useful microbes because they have different microbial community structures compared to surface seawater, although there are fewer organisms. As reported previously[1)], three strains of *Saccharomyces cerevisiae*, which are useful especially in the industry, were isolated and investigated for their phenotypic characterization and were shown to be different compared to the Type strain from the terrestrial environment, such as salt tolerance, cultivating temperature, chronological lifespan and the ability for anti-oxidative stress. In this research, we reported the ability of these strains to produce anti-oxidative stress, which will be investigated further.

Method

After isolated strains and the Type strain were inoculated into YPD culture medium, which was prepared either with purified water (DW) or DSW, they were cultivated until the stationary phase (48 h). After cultivation, each yeast cell was collected and washed with sterilized DW. They were treated with various concentrations of oxygenated water to create oxidative stress (30℃, 1 h). Afterwards, they were washed with sterilized DW to remove oxygenated water and then suspended in sterilized physiological saline. After 10 μL of these suspensions were inoculated on a YPD agar medium, there were cultivated at 27℃. After cultivation, the ability to induce anti-oxidative stress was compared with each strain of yeast based on their growth on the YPD agar medium.

Result and Discussion

Isolated strains showed remarkably higher anti-oxidative stress levels than in the Type strain in DW medium. On the other hand, the anti-oxidative stress of the Type culture improved remarkably from 280 mM to 2,240 mM when cultivated in DSW. Clearly, DSW had some influence on improving the ability of *S. cerevisiae* to create anti-oxidative stress. In order to investigate more closely the mechanism to improve on this ability of *S. cerevisiae*, plans have been made to examine cultivation using surface seawater and artificial seawater. In the case of artificially extending the chronological lifespan of yeast, since it was reported that the yeast's ability to produce anti-oxidative stress has improved as well [2)], we are considering investigating the influence of DSW on the chronological lifespan. Furthermore, plans have been made to examine the influence of DSW on the ability towards creating anti-oxidative stress in a human cell, which is a eukaryotic cell that is similar to yeast.

(1)*Deep Ocean Wat. Res*., 13(2), 30, 2012.
(2) *Biosci. Biotechnol.Biochem*.,73(12),2787-2789, 2009.

14. 飲用深層海水改善醋酸引起之大鼠十二指腸潰瘍是透過硒所產生之抗氧化與抗凋亡保護機轉

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1. 前言:

深層海水是從深度200公尺以下之海水所取出，現已被認為是具有高純度營養成份與礦物質濃度、低溫等特性。海水成份已經被分析含相當高濃度的鎂離子(Mg^{2+})、鈣離子(Ca^{2+})、鉀離子(K^{+})、鈉離子(Na^{+})與微量元素硒，而深層海水因與表層海水相較，具有高度潔淨性，適合應用於生技產品之開發。目前深層海水之生物醫學應用可降低血脂肪所引起之血管硬化、改善異位性皮膚炎等症狀與過敏皮膚等反應。飲用深層海水也可延緩白內障發展與預防動脈血管硬化。 對於深層海水其中之有效成份是相當值得探究。科學證據指出高濃度的鎂離子(Mg^{2+})會影響胃幽門螺旋桿菌感染、胃潰瘍與改善胃食道逆流病徵。另一方面，低攝食抗氧化物如維他命和微量元素硒會促進胃幽門螺旋桿菌感染慢性胃炎從早期快速進展至慢性萎縮胃炎。因此，根據上述，深層海水中之特殊成份可能具有對抗氧化壓力或減低發炎之保護效果。但是，目前現有文獻有關深層海水飲用對於常見醋酸引起之十二指腸潰瘍之效應與機轉仍舊闕如。因此本研究主要在探討不同硬度之深層海水中之內含物、對抗氧化物或漂白劑H_2O_2(過氧化氫)與HOCl (次氯酸)之活性、其氧化還原電位與pH酸鹼值。我們評估十二小時禁水、飲用自來水和硬度1200深層海水之腸道壓力、十二指腸微循環、氧分壓與腸道溫度之變化、以及氧化壓力和細胞凋亡基因之變化。我們假設發炎和氧化壓力會促進腸道老化與十二指腸潰瘍，同時促進十二指腸細胞凋亡。經過一週的飲用不同水樣品，包括深層海水、自來水、含硒水或高鎂離子水，我們利用病理分析與西方墨點法評估醋酸引起十二指腸潰瘍的程度與細胞凋亡程度。我們藉由此實驗要探討深層海水中何種成份可能是提供腸道保護作用之因素，同時也釐清是透過何種機轉減低氧化壓力與細胞凋亡。

2.實驗方法:

我們評估深層海水之 pH，氧化還原電位數值，抗氧化活性，硬度與其礦物質濃度和微量元素硒濃度。我們首次評估 pH，氧化還原電位數值在經過胃或十二指腸之變化。我們利用 OxyLite 2000E plus OxyFlo 系統評估十二小時禁水、飲用自來水和硬度 1200 深層海水之腸道壓力、十二指腸微循環、氧分壓與腸道溫度之變化。我們利用 RNA purification microarrays 分析十二指腸細胞氧化壓力和細胞凋亡基因在面對自來水(TW，硬度：2.48 ppm)，深層海水 600 (硬度:600 ppm)，深層海水 1200(硬度:1200 ppm)之變化。我們亦評估飲用上述水樣品與含高鎂離子[$MgCl_2$ 225 或 450 mg/L]或含硒水[Sodium selenite, 1 mg/L]於雄性 Wistar 大鼠之十貳指腸在面對醋酸刺激造成之潰瘍與細胞凋亡程度。評估方法是病理分析、免疫染色與西方墨點技術。

3. 結果與討論:

深層海水具高 pH 值，低氧化還原電位，高濃度的鎂離子(Mg^{2+})、鈣離子(Ca^{2+})、鉀離子(K^{+})、鈉離子(Na^{+})與微量元素硒. 深層海水具有高能力之清除 H_2O_2 與 HOCl 之能力。飲用深層海水在硬度 600 或 1200 均顯著(P<0.05)上調數種抗氧化與抗凋亡基因，同時也明顯降滴氧化壓力與促進細胞凋亡基因表現。飲用任一種水均會相似明顯(P<0.05)增加腸道壓力、十二指腸微循環、氧分壓與腸道溫度。我們也發現飲用飲用深層海水在硬度 600 或 1200 與含微量元素硒水均顯著降低醋酸造成之十二指腸潰瘍面積與降低細胞凋亡之數量，但含高鎂離子之二次蒸餾水則否。總結，飲用深層海水之腸道保護作用是和微量元素硒上調抗氧化 Thioredoxin reductase 1 基因和抗凋亡 Bcl-2 機轉有關。

14. Deep-seawater Intake Improves Acetic Acid-induced Duodenal Ulcer via Selenium's Antioxidant and Anti-apoptotic Action in Rats

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I. Introduction

Deep sea water (DSW) is characterized by high purity, low temperature, high nutrients and minerals and is obtained from the water flows 200 m under the surface of the sea. Seawater has been identified for containing higher levels of magnesium (Mg^{2+}), calcium (Ca^{2+}), and potassium (K^+) and selenium. DSW being highly clean compared with surface and mid-sea water is superior for biomedical applications for humans as well as for foods and drinks. The biomedical application of DSW in reducing lipid profile and improvement of atherosclerosis, dermatitis syndrome and allergic skin responses has been demonstrated. DSW intake delays cataract development and prevents atherosclerosis. High concentration of Mg^{2+} has been reported to affect *Helicobacter pylori* infection, gastric ulcer and ameliorates gastro-oesophageal reflux disease. It is a fact that diets low in antioxidant vitamins and other micronutrients such as selenium can modulate the conversion of *H. pylori*-associated active chronic gastritis from its early stages to chronic atrophic gastritis. Based on these results, DSW with specific components may have the protective potential against oxidative stress and inflammation. However, data regarding the possible mechanism of the intestinal protective effects of DSW drinking on acetic acid-induced duodenal ulcer have not been reported. In the present study, we have evaluated the ingredients, antioxidant H_2O_2 and HOCl activity, oxidative redox potential (ORP) and pH value *in vitro* in different hardness adjusted by the bittern extracted from DSW. We determined the responses of intestinal hemodynamics, partial pressure of oxygen concentration, temperature and oxidative stress and apoptosis genes profiles to water restriction, TW (tap water), and DSW drinking. We hypothesize inflammation and oxidative stress may enhance apoptotic cell death formation in the duodenal ulcer. After one-week of TW or DSW drinking, we evaluated the intestinal protective effect of DSW drinking in the rat subjected to acetic acid-induced duodenal ulcer by pathologic scoring and western blot. We also compared the effect of waters containing $MgCl_2$ or selenium on acetic acid-induced duodenal ulcer. We suggest that DSW drinking may attenuate reactive oxygen species (ROS)-triggered apoptosis in the duodenal ulcer via its specific components.

II. Materials and Methods

We characterized the pH, ORP value, antioxidant activity, hardness, and concentrations of minerals or micronutrients selenium in the DSW. We explored the responses of pH and ORP value in the stomach or duodenum, the duodenal pressure, oxygenation, and microvascular blood flow by OxyLite 2000E plus OxyFlo systems and analyzed the oxidative stress and apoptosis gene expressions by RNA purification microarrays in the duodenal epithelium in response to tap water (TW, hardness: 2.48 ppm), DSW600 (hardness: 600 ppm) and DSW1200 (hardness: 1200 ppm) infusion in male Wistar rats. We evaluated the effect of DSW, $MgCl_2$ and selenium water drinking on duodenal ulcer by pathologic scoring, immunohistochemical and western blotting techniques.

III. Results and Discussion

DSW is characterized by higher pH value, lower ORP value, higher minerals such as Na^+, K^+, Ca^{2+}, Mg^{2+} and higher micronutrients like selenium. DSW displayed a higher antioxidant H_2O_2 and HOCl activity when compared to TW samples. DSW600 or DSW1200 significantly ($P<0.05$) up-regulated antioxidant and anti-apoptotic genes and down-regulated proapoptotic genes expression vs. TW-treated group. Water infusion significantly ($P < 0.05$) increased intestinal pressure, O_2, and microvascular blood flow to a similar degree in both groups. Drinking DSW600, DSW1200 and selenium water but not Mg^{2+} water significantly attenuated the ulcer area and apoptosis formation in the acetic acid-induced duodenal ulcer.

In conclusion, DSW containing selenium provides intestinal protection via antioxidant and anti-apoptotic mechanisms.

15. 海洋深層水体験施設における長期・継続的な運動浴による健康増進効果の検討

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【目的】

これまで長期・継続的な海洋深層水を用いた運動浴の健康増進効果の検討を行い、本学会において報告してきた。今回は、これまで報告してきた、メタボリック症候群対策への利用、健康関連 QOL の改善および皮膚状態への効果について、まとめて報告する。

【対象および方法】

中高年女性（年齢 40～69 歳）で、腹囲がメタボリック症候群のスクリーニング基準（90cm）以上、または BMI が 25 以上の肥満者 73 名と、基準未満の非肥満者 47 名の合計 120 名を 3 期に分けて募集し、調査対象とした。深層水体験施設「タラソピア」で、3 ヶ月間、週 1 回以上、深層水中（34℃）で歩行浴を主とした運動浴を行った。深層水浴期間の開始時と終了時に、身体測定、血液生化学的検査等の他、健康関連 QOL の SF-36v2™ による調査および皮膚状態に関する調査を行った。皮膚状態への季節的影響の検討のため、2 期目と 3 期目については、互いに対照群になるように運動浴を行わないときの皮膚状態の調査を追加した。本研究は、富山県衛生研究所倫理審査委員会の承認を受けて実施した。

【結果および考察】

終了時の調査に参加した肥満者 63 名、非肥満者 42 名（合計 105 名、対象者の 88%）について検討した。3 ヶ月間の深層水浴回数は、平均 30 回であった。深層水浴期間前後の身体状況の変化をみると、肥満者において体重および腹囲が有意に減少し、非肥満者においても腹囲の減少がみられた。また、肥満者と非肥満者のいずれにおいても、血中の HDL-コレステロールが有意に増加し、HbA1c の有意な減少がみられ、深層水体験施設における 3 ヶ月間の運動浴が、肥満や生化学的検査の指標を改善し、メタボリック症候群対策に有効であることが示唆された。

深層水浴期間前後の QOL スコアを比較すると、全体としてほとんどの健康尺度およびサマリースコアが上昇した。最も上昇したのが、精神的健康の尺度である VT（活力）と身体的健康の尺度の GH（全体的健康感）であり、続いて身体的健康の BP（体の痛み）、PF（身体機能）、RP（日常役割機能）および精神的健康の MH（心の健康）が上昇し、サマリースコアである MCS（精神的側面）と PCS（身体的側面）も上昇した。肥満者は、開始時に非肥満者に比べて、PF、GH、VT、MH、MCS が有意に低かったが、終了時には GH と PCS 以外は差がみられなくなり、肥満者の改善傾向がみられた。

3 ヶ月間の深層水浴後の皮膚状態のアンケート調査では、105 名中 49 名から「しっとりした」「かさかさしなくなった」など「よくなった」との回答があったが、皮膚状態の測定指標とした前腕部および腹部の水分蒸散量、角質水分量および pH は、9～12 月の深層水浴実施群において悪化傾向を示した。深層水浴を実施しない対照群においても同様の測定時期による変動がみられ、季節的影響によるものと考えられた。これらの皮膚状態の測定指標に関して、深層水運動浴の有無による調査期間前後の変化の違いについて検討したが、深層水運動浴によって変化のパターンが異なるとは言えなかった。今後、新たな皮膚関連の測定項目を追加するなどさらに検討を加えて行きたい。

【まとめ】

メタボリック症候群対策として深層水を利用した長期・継続的な運動浴が有効であることが示唆された。3 ヶ月間の深層水運動浴前後の比較で QOL スコアの上昇がみられた。皮膚状態のアンケート調査では、改善の感想が多くあったが、測定指標では深層水運動浴による変化はみられず、さらに検討を行う。

15. Studies of Health Effects Using Long-Term and Continuous Exercise Bathing in DSW Plant

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Objective

The aim of this paper was to report our studies of health effects on preventive measures against metabolic syndrome, health related quality of life (QOL) and skin condition using long-term and continuous exercise bathing in deep sea water (DSW) plant.

Subjects and Methods

120 women aged from 40 to 69 years (73 fat women with waist circumference (≧90cm) or body mass index (≧25) and 47 non-fat women) were recruited 3 times separately. The subjects had exercise bathing mainly walking in DSW of 34 ℃ more than once a week for 3 months. Before and after the 3 months' exercise bathing, body measurement, biochemical blood test, QOL of SF36v2™ questionnaire and skin condition were examined. To estimate for seasonal influence on skin, additional examinations of skin condition were conducted when not in DSW bathing in 2nd and 3rd term groups. These studies were done with the approval of the Ethical Review Board of the Toyama Institute of Health.

Results and Discussion

63 fat and 42 non-fat women (total 105 persons, 88% of subjects) who participated in end investigation were examined. The frequency of exercise bathing for 3 months had the average of 30 times. After the 3 months' exercise bathing, weight and waist circumference decreased significantly in fat women, and waist circumference decreased significantly in non-fat women. Moreover, HDL-cholesterol in serum increased significantly, and HbA1c reduced significantly in both fat women and non-fat women. It was suggested that the 3 months' exercise bathing in DSW is effective against metabolic syndrome.

After the 3 months' exercise bathing, VT (vitality) and GH (general health) of QOL health subscales in participants increased most, then BP (bodily pain), PF (physical functioning), RP (role physical) and MH (mental health) increased, and MCS (mental summary score) and PCS (physical summary score) also increased. Although fat women had low scores of PF, GH, VT, MH and MCS compared with non-fat women at the starting time, no differences were seen at the end time except GH and PCS.

There were many replies of "it became good" such as "it carried out gently" and "not carrying out restfully" from 49 persons among 105 participants in questionnaires of skin condition after the 3 months' exercise, but the measured index of skin condition (such as transepidermal water loss, stratum corneum moisture and skin surface pH at forearm and abdomen) were thought to be aggravated during examination period from September to December and to be influenced by season. About the index of skin condition, no significant interaction was seen between exercise bathing and the measurement time. A further study for skin condition by DSW bathing is needed.

16.深層水細菌の生理活性化合物とその利用に向けた進展

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1. はじめに

各地で海洋深層水がエネルギー資源、水産資源、食品原料、化粧品原料等として利用されている。一方、海洋深層水中から分離された微生物が産生する酵素や生理活性化合物を利用することも試みられている。しかし、このような微生物の生産物を実用的に利用しようとする取り組みには、生産物の特性解明に加えて、生産性向上と精製法の改良による供給量の確保及びコスト低減が極めて重要となる。本演題では、深層水細菌が産生する生理活性色素ヴィオラセインを例としてその利用に向けた取組みについて報告する。

2. 方法

海洋深層水は高知県海洋深層水研究所の採水施設で採取した。色素の化学構造は質量分析とNMRによって解析した。

3. 結果

深層水中より分離された細菌である *Pseudoalteromonas* sp. 520P1株は青紫色素を産生した。この色素の質量分析とNMR解析により、これをヴィオラセイン(violacein)と同定した。ヴィオラセインは陸上細菌の *Chromobacterium violaceum* や *Janthinobacterium lividum* の他、*Pseudoalteromonas* 属の海洋細菌が産生する抗菌性色素である。また、この色素が抗菌性だけでなく、白血病細胞HL60及びU937に対して細胞毒性を示すことを見出した。このようにヴィオラセインには生理活性化合物としての利用価値があると考えられた。

しかし､520P1株によるヴィオラセインの生合成は、細菌の遺伝子発現調節機構であるQuorum Sensing (QS)の制御下にあり、ヴィオラセイン生産には複雑な培養条件が必要であることが分かった。そのため、520P1株は工業的なヴィオラセイン生産には適していないと考えられた。

そこで、QSによる発現制御を回避するため、520P1株ゲノムDNAライブラリーからヴィオラセイン合成酵素遺伝子群を単離した。これをpETベクターに組込み、大腸菌内でヴィオラセインを合成させることに成功した。一方、QS機構に変異を生じたと考えられる520P1変異株を分離した。この変異株は通常の振とう培養法で容易にヴィオラセインを産生した。組換え大腸菌又は変異株を用いると、培養時間を従来の7～10日間から1～2日間に短縮できる上、ヴィオラセインの収量は約3倍に向上した。また、得られたヴィオラセインを簡便な方法で大量精製することにも成功した。

4. 考察

微生物から有用化合物を見出したとしても、生産量とコストが隘路となり、実用化に至らない場合も多い。本研究ではこれらの課題を乗り越えることができたと考える。現在は企業や大学へヴィオラセインをサンプルとして提供している。しかし、ヴィオラセイン等の化合物の市場への導入には、化合物の生産を行い、生産物を製品化する企業が重要なステークホルダーとして参入することが必須と考えられる。

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16.Bioactive compounds of bacteria isolated from deep ocean water and the recent progress for their utilization

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1. Introduction

Deep ocean water has been used as energy resources, marine resources, food materials, and cosmetic materials. It has been also tried to use enzymes and bioactive compounds produced by microbes isolated from deep ocean water. However, it is essentially important for practical use of microbial products to ensure the supply and reduce the cost by improving productivity and purification methods. We report here our study toward the utilization of violacein, a bioactive pigment produced by bacteria isolated from deep ocean water.

2. Methods

Water sampling of deep ocean water was conducted at the Kochi prefectural deep seawater laboratory. The chemical structure of the pigment was analyzed by mass spectrometry and NMR.

3. Results

Pseudoalteromonas sp. strain 520P1, the bacterium isolated from deep ocean water, produced a blue-purple pigment. This pigment was identified as violacein, using mass spectrometry and NMR. Violacein is an antibacterial pigment produced by terrestrial bacteria including *Chromobacterium violaceum* and *Janthinobacterium lividum* and marine bacteria belonging to genus *Pseudoalteromonas*. In addition to the antibiotic activity, we found that this pigment showed cytotoxicity to leukemia cell lines HL60 and U937. Thus, violacein was considered to be useful as a physiologically active compound.

Violacein production however required complicated culture conditions because violacein biosynthesis by strain 520P1 was under the control of Quorum Sensing (QS), the regulatory mechanism of gene expression in bacteria. Strain 520P1 therefore seemed unsuitable for industrial violacein production.

In order to avoid control by QS, an enzyme gene cluster responsible for violacein synthesis was isolated from the genomic DNA library of 520P. Then the gene cluster was inserted into pET vector, and expressed successfully in *E. coli*. On the other hand, we isolated a variant strain of 520P1 that is considered to have a mutation in QS mechanism. This variant produced violacein easily by conventional shaking-culture methods. Using recombinant *E. coli* and the variant, culture time was shortened to 1-2 d from 7-10 d when strain 520P1 was used. The yield of violacein was also improved by about 3-folds. Moreover, large-scale purification of violacein was achieved by a simpler method.

4. Discussion

Even if a useful compound was found in microbes, it does not result in utilization in many cases because of insufficient quantity and the high cost of production. In this research, we were able to overcome most of these problems and are supplying violacein samples to industries and research institutes. However, to introduce compounds including violacein into the market, the participation of companies that produce compounds and use them for industrial products is indispensable as important stakeholders.

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17. 海洋深層水飲用による腫瘍細胞増殖抑制効果の関与蛋白解明

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【目的】

これまで本学会にて、海洋深層水より調製した高ミネラル水は、循環器系疾患の改善、ピロリ菌の増殖抑制や腫瘍細胞の増殖抑制など健康に有益な効果を有する事を報告してきた。現在は、腫瘍細胞の増殖抑制機序解明を目的に解析を行っている。本報告では、高ミネラル水による腫瘍細胞増殖抑制効果に関与している蛋白の検索経過を報告する。

【方法】

高知県室戸沖より採水した海洋深層水を用いて、調製した 5 種類の高ミネラル水(マグネシウム/カルシウム(mg/L)、A：100/200、B:150/150、C:200/70、D:240/0、E：0/350)を試験飲料として用い、コントロールには精製水を用いた。これらの飲料をヌードマウス(雄、4 週齢)に 2 週間自由摂取後に腫瘍細胞(ヒト胃ガン由来細胞：以下 MKN-45 または、ヒト子宮ガン由来細胞:以下 Hela)を後頸部皮下に 1×10^7 細胞移植した。腫瘍細胞移植後も各飲料の自由摂取を継続させ、移植 2 週間後に腫瘍と血清を採取した。腫瘍は超音波破砕後、採取した各血清と抗マウス IgG 抗体を用いてウェスタンブロット(2 次元電気泳動)を実施し、コントロール群と高ミネラル水摂取群間で抗体反応プロファイルを比較した。

1 次ステップは両群間で差を認めた箇所のゲルを LC-MS/MS に供し、腫瘍細胞増殖抑制効果に関与する可能性のある候補遺伝子を抽出した。

2 次ステップは、mRNA を用いて得られた候補遺伝子の cDNA を作成し、それらの全遺伝子に対して His 融合蛋白産生用の plasmid を分子生物学的手法で構築し、各融合蛋白を大腸菌で誘導発現後、1 次ステップと同様に各血清との抗体反応性を解析し各候補蛋白を検証した。

3 次ステップでは、抗体反応性の結果、絞り込んだ候補遺伝子の cDNA から FLAG 融合蛋白産生用の plasmid を構築し、各融合蛋白を腫瘍細胞内で発現させ、腫瘍細胞の viability を解析している。

【結果・考察】

腫瘍サイズは MKN-45 と Hela の両腫瘍細胞共にコントロール群と比較して高ミネラル水摂取群で有意に小さかった。抗体反応プロファイルの結果から、高ミネラル水摂取群の腫瘍は、MKN-45 で 50kDa 付近に、Hela は 50kDa と 30kDa 付近にコントロール群では認められないバンドを認めた。1 次ステップでは、MKN-45：44 種類、Hela：31 種類の高ミネラル水の飲用による腫瘍細胞増殖抑制効果に関わる候補蛋白が得られた。2 次ステップ終了時には、MKN-45：9 種類、Hela：5 種類までに候補蛋白を絞り込むことができ、現在は各候補蛋白を腫瘍細胞に導入発現させ、細胞増殖に及ぼす影響について評価・解析している。

17. Elucidation of proteins involved in tumor growth suppression induced by drinking Refined Deep-Seawater

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1. Introduction

We reported earlier the potential effects of refined deep-seawater (RDSW), a kind of high-mineral drinking water, on human health. Drinking RDSW improved cardiovascular disease and suppressed the colonization of *H. pylori* in the stomach and tumor growth *in vitro* and *in vivo*. The final goal of this study is to elucidate the mechanism in which drinking RDSW suppresses the tumor growth. Now we are identifying the proteins involved in tumor growth suppression induced by drinking RDSW and progress is reported herein.

2. Materials and Methods

RDSW was prepared from deep-seawater off Muroto in Kochi. The deep-seawater was conditioned to give 5 types of magnesium/ calcium (mg/L) ratios of A : 100/200, B : 150/150, C : 200/70, D : 240/0 and E : 0/350. The RDSW and distilled water were used as test and control waters, respectively.

Nude mice (male, 4-week-old) were allowed free access to RDSW or control water for 2 weeks. Then, 1×10^7 of each tumor cell (Human gastric cancer cell: MKN-45 or Human uterine cancer cell: Hela) was injected in the subcutaneous part of the posterior cervical region. The injected mice continuously accessed RDSW and control waters. Two weeks later, the animals were sacrificed and tumors and sera were obtained.

All tumors homogenized by ultrasonic device and sera were subjected to western blotting (two dimensional electrophoresis) to evaluate the immuno-reactivity and the antibody profile was compared between RDSW and control groups. The protocols are below.

1st step, based on the antibody profiling, the proteins whose profile differed between two groups were subjected to LC-MS/MS analysis.

2nd step, cDNAs of candidate genes from LC-MS/MS analysis were prepared based on mRNAs and used to make a plasmid which constructs each gene-His fusion protein. All fusion proteins expressed in *E. coli* were subjected to western blotting to evaluate immuno-reactivity according to 1st step.

3rd step, cDNAs of candidate genes from 2nd step were used to make a plasmid which constructs each gene-FLAG fusion protein. We evaluate the cell viability of tumor cells transfected with each plasmid.

3. Results

Tumors (MKN-45 and Hela) grown in mice were significantly smaller in RDSW group compared to control group. Antibody profile showed that ca. 50 kDa (MKN-45 and Hela) and ca. 30 kDa (Hela) proteins were found in only RDSW group. At 1st step, 44 (MKN-45) and 22 (Hela) genes were candidates and could be narrowed down to 9 and 5 after 2nd step. Now we are making the transfectants based on MKN-45 and Hela cells to identify the proteins involved in tumor growth suppression induced by drinking RDSW.

一般講演４
〈海利活用システム関連他１〉

Session 4: Resource Applications 1

座長　　山田　勝久
（株式会社 ディーエイチシー 海洋深層水研究所　所長
東京海洋大学共同研究員）

Chairman: Katsuhisa Yamada
(Director of Department of research in the deep seawater, DHC Corporation,
Joint research member in Tokyo University of Marine Science and Technology)

18. 日本の海洋深層水の調査研究テーマの変遷と利活用の課題

○長野　章[1]、伊藤　敏朗[2]、武下　久恵[2]
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1．はじめに

日本には海洋深層の取水施設が16施設ある。この施設が全国に分布しており、取水規模、利活用の用途及び利用における調査研究連携先など多様な様態を示している。利用目的については水産への利活用が多い。また、水産庁による補助施設が出来てから15年を経ているが、海洋深層水利用に関する調査研究テーマもその時々のニーズに従い変遷している。日本の海洋深層水取水施設の概要と利活用に関する調査研究テーマの変遷と、水産利用を目的として整備された施設の課題を調べた。

2．日本の海洋深層水利活用施設の特徴

日本の海洋深層水の利用目的は水産利用が大半である。しかし、取水量のうちの少量であるが、機能物質の抽出、飲用水、タラソテラピー、その他利用も行われている。水産利用目的は多くの水量を使用すること、また、取水施設整備に水産庁の補助金を充てることが出来る。そのため、日取水量1,000トンを超える取水施設は、水産庁の補助金により整備した取水施設は9施設になる。しかし、取水量の1/2以上は水産利用目的に拘束されるが、その他の用途は限定されていないことにより、多様な用途に利用されている。このことは日本での海洋深層水利活用の発展に寄与している。

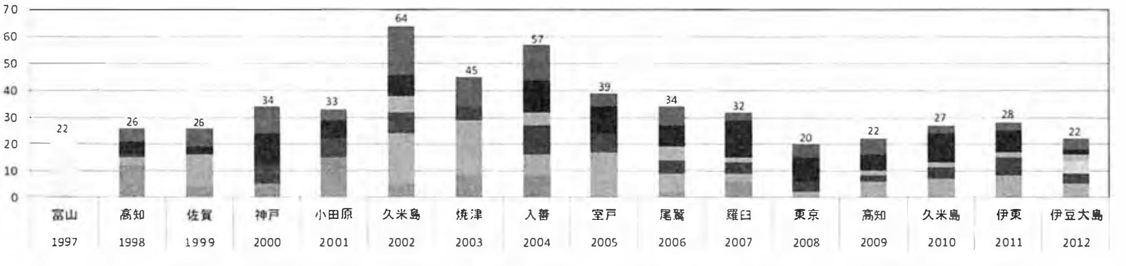
3．調査研究テーマの変遷

海洋深層水に関する調査研究テーマを毎年開催される海洋深層水利用学会の発表セッションの分類を基本に研究発表テーマから調べた。発表セッションは、海洋水質、海洋水産、農畜産、健康医療、利活用システム及び取水施設で、特に2012年から生物化学工学の7つに分類した。結果は図－1の通りである。初期の段階では取水施設が多いが、多いのは海洋水質及び水産関連であり、農畜産及び健康医療関係は少ない。近年は、温度差発電、経済性の追求及び水産生物生産システムに関すること及び水質、水産を根源から調査研究する生物化学工学に関するテーマが増加している。

4．海洋深層水の利用状況と課題

日本の海洋深層水の利用では、水産目的以外の利用は一部でしか産業化されておらず、近年低調になっている。一方、日本では、漁港の衛生環境の高度化を推進している。海洋深層水の3つの利点である雑菌が少なく清浄性に優れており、また水温も低いので雑菌の繁殖などの防止にもなることある。この様な単純な海洋深層水の性質に着目した利活用がなされ、その傍ら、水産物の加工分野への拡大、清浄性のほか低温性に着目した水産生物の幼稚仔の育成等への利用について産業化への普及が見込める。

日本においてあらたな深層水取水施設の整備はなされていない。日本の水産関係者の中では、海洋深層水への興味が薄れている。水産庁、地方公共団体及び民間企業と大学や公設研究機関が一体となった調査研究の推進が必要である。



18. Changing research themes and future applications of deep sea water

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([1]All Japan Fishing Port Construction Association,[2]Fishery Agency, Ministry of Agriculture, Forestry and Fisheries)

1. Introduction

There are 16 deep seawater (DSW) intake facilities in Japan that benefit the fishery industries. It has been 15 years since subsidies system were established with the Fishery Agency of the Ministry of Agriculture, Forestry and Fisheries (MAFF). DSW research has depended on social and industrial needs. An overview about intake facilities and traditional research theme and recent changes are presented in this paper.

2. The DSW facility and its utilization in Japan

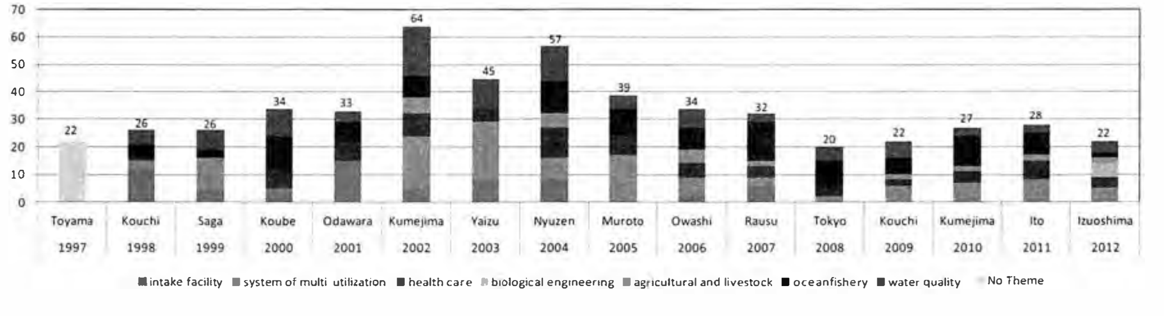
The extraction of functional materials, drinking water and thalassotherapy from DSW occurs in small amounts. The wash water of fish wholesale market, fresh fish and fish processing facilities use a lot of DSW. Intake facilities for fishery purposes are assisted by the Fishery Agency. Nine intake facilities producing over 1,000ton per day were added by the Agency. Therefore the fishery usage is about half, while the rest is not limited. For this reason, DSW has a variety of applications, hence it is promoted in Japan.

3. Tradition of research theme of deep seawater

The DSW research theme presented annually in the conference of the Deep Ocean Water Application Society are classified to 6 items such as water quality, ocean fishery, agricultural and livestock, health care, system of multi utilization and intake facilities (Fig-1). At the start, there were many works about intake facility, water quality and ocean fishery, and a little on agricultural and livestock and health care. In recent years, work on thermal energy, economic efficiency, fishery production system and biological engineering became the fundamental study of DSW.

4. Challenges in the use of DSW

DSW has three advantageous points namely, cleanliness, low temperature and plenty of inorganic nutrient salts. These features of DSW should be exploited in fishery processing and development of fish culture. Fishery officials are not directly interested in DSW. However, fishery agency, local governments, research institutions, private sector and graduate institutes should conduct collaborative research.



19. 富山湾深層水を活用した商品開発の大学における事例

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１．はじめに

1997年以来、富山県立大学では産官学連携推進事業の一貫として、富山湾深層水の非水産分野の有効利用研究に参画し、60品目以上の商品を開発した。開発した商品あるいは農産物の収量増加と品質改善の事例は、食品、アルコール飲料、化粧品と農産物用の葉面散布剤など多岐に渉っている。本報告では、食品ではかまぼこチツプス、アルコール飲料並びに化粧品について、それらの開発過程における深層水の活用法を中心に述べる。

２．かまぼこチツプス

かまぼこは、原料が魚肉で日本や富山の伝統的食材で、しかも高タンパク、低脂肪でヘルシーな食品である。しかし、近年の食文化が洋風化へと大きく変化しつつあるため、かまぼこの消費も年々減少の傾向を示すようになった。このような消費動向から脱するため、幼児、子供、成人、老人に幅広く食していただく新しいタイプの商品の開発が急務となった。開発に当つては、かまぼこの食材を損なうことなく、洋風化にマツチした新しい商品になるよう工夫が必要となろう。また、手軽に食べられるものでなくてはならない。これらの要素を満足させるとするなら、かまぼこのチツプス化が最適と判断した。

しかし、既存のかまぼこをスライスしフライ処理してもパリパリ感やふんわり感が得られなかった。この問題の解決のため、開発に当って27回の試行錯誤を繰り返し、魚肉すり身、タピオカでんぷん、米粉、調味料などを富山湾深層水を用いて練り上げ、1～2mmの厚さに成形、乾燥後、フライ化あるいは焼入れ処理する方法を確立した。さらに、風味の幅を広げるため、種々の素材の添加も可能にした。現在、商品としては、プレーン、昆布味、白えび味、ワサビ味とマヨネーズ味の5種類を市販している。なお、この商品には、日本国特許（第5235929号）が与えられた。

３．アルコール飲料

地ビールは、地域独特のこだわり商品として各地で数多く製造販売されている。富山県でもいち早く氷見市の㈱いきいき地ビールと富山県立大学が共同でこれに取り組み、富山湾深層水を0.1～0.8%加えた仕込み用水で二条大麦の麦芽を発酵させた。その結果、24時間、20℃のフラスコ培養条件でのビール酵母の増殖は、深層水を0.8%添加した区で対照区に比べて約10倍高くなった。また、ビール醸造過程で深層水を0.8%添加したところ、発酵4日目からアルコール濃度が対照区に比べ明らかに高くなり、6日目のアルコール濃度は、対照区に比べ0.5%～1.0%高かった。一方、高アルコール濃度ビールの製造では、糖化工程の仕込み水に深層水を0.4%添加し、17.5～18.8プラートの麦汁にビール酵母を添加しアルコール発酵させると、発酵8日目でアルコール濃度8%のビールが得られた。これらのビール製造方法で、日本国特許を取得した（第3631995号）。商品としては、めざめるビール（アルコール濃度5.5%）と波奏（わかな）麦酒（アルコール濃度8%）をそれぞれ販売した。

他方、焼酎の製造では、コシヒカリ白米を原料に、黄麹7号で種こうじをつくり、糖化行程では、一次もろみを、種こうじ、二条大麦，深層水＋地下水および酵母を混合して5～7日発酵させ、さらに二次もろみを3週間発酵させた後、ろ過═と単式蒸留を行ったところ、アルコール濃度39.5%の原酒が得られた。次に、原酒の熟成を早めるため低超音波（40KHZ, 12mW）処理を実施した。この処理では、エタノール分子と水分子の融合が促進されることを^{1}HNMRＲスペクトルで確認した。この方法によれば、3年余りの原酒の熟成期間が必ずしも必要ない。商品は、黒部（アルコール濃度25%）と譲（アルコール濃度30%、高峰譲吉博士にちなんで）の2種類である。

４．化粧品

珪酸を主成分とする石英斑岩の微粉末を調製し、それを用いて深層水で石英斑岩ゲル化物を製造することを大きな特徴としている。このゲル化合物は、Fun-A-Oreと名付け石鹸やクリームの素材にした。このゲル化合物を用いたラット皮膚熱傷モデルの治癒促進効果試験、並びに「Fun`Preseしっとりソープ（ゲル化合物9.1%含有)」を用いたモルモット皮膚炎症モデルの活性酸素除去試験を実施したところ、いずれも優れた効果が得られた。さらに、後者の石鹸とゲル化合物配合のスキンクリームと併用するとアトピー性皮膚炎に改善効果を示した。また、抗菌活性のあるティーツリーオイルを含むFun`Prese石鹸は、水虫治療に効果が認められた。2012年には、生体機能活性化物質の名称で日本国特許を取得した（第4799724)。

謝辞：　地ビール製造では㈱いきいき地ビールに、焼酎製造では銀盤酒造㈱に、並びに化粧品製造では㈲リットライフにそれぞれお世話になった、深く感謝申し上げます。

19. New Goods Produced by Using Deep Sea Water in Toyama Bay

——An Example of University Laboratory——

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1. Introduction

In 1997, the non-fishery field project involving the effective use of deep sea water in Toyama bay was started. At the present time, new goods produced by Toyama Prefectural University (especially in Drs. Furumai and Yoshida laboratories) were more than 60 items. Our interests were focused on agricultural products use for quality and quantity improvement, foods, alcoholic beverages and cosmetics. We should be noted here that the concept for the good development is one item per company and first come, first service. In the present paper, we would like to introduce about the kamaboco-chips produced from fish meat paste, beer and cosmetics. All goods were supported by the additive effect of deep sea water.

2. Kamaboco-chips

The traditional Kamaboko (boiled fish paste) is composed of fish meat, potato starch, and salt. However, western-style-izing of eating habits in Japanese causes a decrease of personal consumption of Kamaboco, especially in young generation. This social phenomenon is proposed that an increase of consumption is necessary to develop a new-type Kamaboco. Therefore, breakthrough has been made by the combination of fish meat, deep sea water, rice powder, tapioca powder and monosodium glutamate. The chips (1~1.5mm thickness) were completed according to deep fry or roast. Now, 5 items are on sale.

3. Alcohol beverages

In the case of beer and shochu (Japanese spirits) production, the acceleration of yeast growth was the most important factor for the alcohol fermentation. In this project, the alcohol content in barley malt liquid or shochu malt liquid was accelerated by the presence of deep sea water. Additional rate of deep sea water was 0.4% to 0.8 %. On the other hand, the ripeness of shochu was stimulated by using the supersonic wave (40 KHz,12 mW).

4. Cosmetics

One of mineral matter for cosmetics is quartz porphyry. This objective was to develop the new functional cosmetics by using the super powder of quartz porphyry. As a result, the gel compound (named Fun-A-Ore) with deep sea water and the soap containing its gel compound gave evidence for the positive effect to Rat skin-heat injury test. The soap (Fun`Prese moist soap) also has the removing effect from guinea pig skin of active oxygen. Thus, the gel compound of quartz porphyry with deep sea water acts as an anti-oxidant.

From results obtained here, we would like to conclude that the deep sea water in Toyama bay was very useful to develop foods, alcohol beverages and cosmetics for new style.

20. 臺東大學深層海水產業產學合作建構之展望

º劉炯錫(臺東大學)、王文清(能高休閒育樂公司)、劉金源(臺東大學)、徐享崑(能高休閒育樂公司)、
陳小琪、段文宏、陳雲芳、馮千芝、李俊霖(臺東大學)、張禎祐(臺東專科學校)、
陳芝融、陳孟炬、楊春桂(臺東大學)

1.前言

深層海水產業在日本於 1990 年代已從高知縣等地逐漸發展開來。臺灣民間業者在 2005 年起自行於花蓮市取水並生產相關產品。經濟部在 2007 年選定臺東縣知本溪口作為深層海水低溫利用及多目標技術研發模廠，以打造臺東市成為深層海水產業重鎮。台東大學生命科學系李俊霖副教授自 2008 年起在國科會支持下，在深層海水於生物技術應用研究上，已證實深層海水可促進紅麴菌與樟芝菌之生長與功能代謝物生成，並提升紅麴降血脂效果與樟芝護肝之效果，此兩篇研究已分別發表於 *Journal of agricultural and food chemistry*.與 *Applied Microbiology and Biotechnology* 之 SCI 期刊上。

2.發展現況

臺東大學於 2010 年 5 月成立深層海水產業科技研發中心後，在經濟部深層海水模廠旁投資渡假村的能高休閒育樂(股)公司主動尋求合作，協助本校在 2011 年 9 月設置綠色科技產業碩士班，並開設深層海水產業利用特論等課程，2013 年 7 月已有林締怡、林政佑、鄭又升、李家瑩、古馥維等五位同學分別以國外旅客、冷能利用、雞松茸培養、海洋運動休閒及原住民議題等與深層海水直接或間接相關的題目，完成碩士論文，並於今年 9 月進入該公司服務；另預計 2014 年也有 5 篇相關碩士論文發表。

2013 年 6 月起，本校理工學院結合臺東專科學校，並取得深層海水製品(股)公司、能高休閒育樂(股)公司、富洋國際開發(有)公司、菜籃子生物科技(有)公司、晉安製藥(股)公司合作意願書，而獲得經濟部技術處學界科專「臺東深層海水產業關鍵技術研發三年計畫」，開始穩定累積研發基礎之實力，包括建立辦公室、網站、專業文獻資料庫、專利與技轉服務能力、產業輔導能力、產業論述規劃能力，以及產官學研平台外，配合劉金源校長水下技術之專長，特別邀請國內外學者專家論述發展取水相關技術。另李俊霖副教授主持「應用 DSW 與其功效離子開發具降低體脂肪功能之乳酸菌發酵製品」計畫，段文宏助理教授「以深層海水開發培育微藻生產天然抗氧化劑技術」，陳芝融助理教授研究「深層海水梅子發酵酒之製程研發與機能性成份分析」，陳孟炬助理教授評估「冷能空調利用」，張禎祐教授主持「海洋深層水於蔬果、肉品及漁獲保鮮之研究」等，均按既定進度順利進行中。本校創新育成中心並利用深層海水研發成果製作招商手冊，預期在第一年即有產業成果。

3.未來展望

本校除上述產學合作計畫外，擬視本校研發成果與產業市場需要，擴大與企業合作培養人才，持續每年開設深層海水產業特論課程，並在 2014 學年度設置深層海水產業碩士班，以為臺東深層海水產業園區培養經營與研發人才，兼作為成為臺東地區深層海水產業的論述基地與交流平台。

20. Prospects for University-Industry Collaborations Between the Deep Sea Water Industry and National Taitung University

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([1]National Taitung University, [2]Neng-Gao Entertainment Corporation, [3]National Taitung College)

I. Introduction

The Deep Sea Water (DSW) industry in Japan started in Kochi in the 1990's. In Taiwan, water pumping systems were installed in Hualien by private enterprises and DSW products were produced in 2005. The Ministry of Economic Affairs (MOEA) in Taiwan decided to construct a low temperature sea water and multipurpose technical R&D model plant in Taitung in 2007. Taitung has now become one of the significant centers of the DSW industry. Supported by the National Science Council since 2008, Prof. Lee of the National Taitung University (NTTU) found that DSW accelerated the growth of *Monascus* sp. and *Antrodia camphorota* and the formation of functional metabolites. DSW also accelerated hypolipidemic effect of *Monascus* sp. and the liver protection effect of *A. camphorota*. These two papers have been published in two SCI journals, *Journal of Agricultural and Food Chemistry*, and *Applied Microbiology and Biotechnology*.

II. Development Status

After NTTU established the DSW R&D Center in May 2010, Neng-Gao Entertain- ment Corp. started the cooperation with NTTU and assisted in establishing the green technology industry master program in Aug, 2011. T.Y. Lin, Z.Y. Lin, Y.S. Cheng, C.Y. Lee, and F.W. Gu studying foreign tourists, thermal energy utilization, *Agaricus blazei*, coastal recreational activities, and indigenous people's issues, respectively, finished their master's theses and will work in the Neng-Gao Entertainment Corp. in Sept. 2013. In addition, five master's theses relating to DSW will be presented in 2014.

Since June 2013, NTTU has cooperated with the National Taitung College, and signed letters of intention with DSW Production Co. Ltd., Neng-Gao Entertainment Corp., Rich Gate International Ltd., National Basket Co. Ltd., and Chin-Ang Pharmaceutical Co., Ltd. Supported by the Technology Development Program for Academia of the MOEA, NTTU has made an effort under the 3-year-research project on key technologies of DSW in Taitung and has acquired the abilities for research and development including project office, website, database, patent and technology transfer service, industrial counsel, planning abilities and information exchange platform for industry, government, universities, and research organizations. Besides, President J.Y. Liu held a conference and invited local and international experts to discuss water pumping technology. The following are ongoing DSW projects- "Using DSW and its ion develop lactic acid bacteria fermented products that reduces body fat" by Dr. C.-L. Lee, "Technology for producing natural antioxidants from microalgae cultured in DSW" by Dr. W.-H. Twan, "Manufacturing process study and functional ingredients analysis of DSW plum-wine" by Dr. C.-R. Chen, "Cold energy utilization in air conditioning" by Dr. M.-C. Chen and "Research on using DSW in vegetables, meat, and fishery preservation" by Dr. C.-Y. Chang. Based on the above-mentioned projects, the NTTU Innovation & Incubator Center made a DSW industry investment brochure.

III. Prospect

NTTU will keep expanding the collaboration with enterprises for developing talents on the basis of research effort and market demand, and keep offering DSW industry courses. NTTU will start on the DSW industry master program in 2014 to train management and research talents for the Taitung DSW industry. It is expected that NTTU could become the center to generate knowledge and to exchange information and technologies within the Taitung DSW industry.

21. 利用奈過濾系統製備高鈣鎂比與脫硫之深層海水礦物質液

°黃育楓、駱呈欣、詹張灝、黃秉益(石資中心)

一、前言

深層海水具有低溫、富含礦物質、水質潔淨、大量與安定之特性。雖然深層海水中擁有豐富的礦物質含量，但值得注意的是同時含有對人體有害的硫酸根離子。硫酸根離子亦會與鈣離子形成硫酸鈣沉澱，造成應用過程中鈣離子含量的下降以及設備鍋垢的問題。因此，如何有效去除深層海水中的硫酸根是產業應用上重要的研究課題。目前國外商業化的深層海水脫硫製程多採為電透析技術，但此方法對以中小企業為主的台灣而言，操作成本過高以及操作範圍的限制造成投入之瓶頸。為了有效降低操作成本，近年來已有許多研究利用薄膜分離方法去除海水中的硫酸根離子。然而，雖然使用薄膜分離方法可有效去除硫酸根離子並降低操作成本，但值得注意的是此技術在脫硫的同時亦會造成深層海水中礦物質成分的流失。在此基礎下，本研究擬嘗試建立一套多段式奈米膜過濾系統，並藉由調控過濾路徑以及操作條件的方法來有效去除深層海水中硫酸根離子並同時保留對人體有益的礦物質。

二、實驗方法與步驟

本研究利用經濟部東部深層海水創新研發中心所建置之奈過濾膜分水設備進行脫硫實驗。並以感應耦合電漿質譜儀與離子層析儀測定產物與原水之鈣、鎂濃度與硫酸根等濃度。

此過濾系統分為兩階段，第一階段(NF1)先進行深層海水濃縮或淡化步驟，再將第一階段所生產之濃水或淡水作為第二階段(NF2)之進料水，並比較各種不同過濾路徑所產出產物之鈣、鎂離子保留率及硫酸根離子之脫除率，以獲得最佳化之過濾路徑。在獲得最佳化過濾路徑後，本研究進一步調整各階段奈過濾系統之操作壓力，並分析所取樣品之鈣、鎂離子保留率及硫酸根離子去除率，以獲得最佳化之硫酸根離子去除以及鈣、鎂離子保留的操作條件。

三、結果與討論

本研究成功利用多段式奈過濾系統有效的去除深層海水內之硫酸根離子並同時保持深層海水中對人體有益的鈣、鎂離子等礦物質。由實驗結果可知，過濾路徑與操作壓力會影響所獲產物之鈣、鎂離子保留率與硫酸率去除率。當使用路徑取淡+提濃(P+C)路徑進行過濾程序時，所獲得產物之硫酸根離子去除率（62.2%）明顯低於另一過濾路徑提濃+取淡(C + P)所獲得產物（98.8%）。由此可知，路徑 P+C 較不適合用於去除深層海水中的硫酸根離子。在此條件下，本研究利用路徑 C + P 來去除深層海水中的硫酸根離子，並進一步透過增加奈過濾系統操作壓力的方式來提升產物內之鈣、鎂離子濃度。

由實驗結果可知，當 NF1 系統與 NF2 系統的操作壓力皆達到 150 psi 時，相對於原水，產物內鈣離子與鎂離子的保留率可分別達到 72.5%和 34%。此外，由實驗結果可知，經此過濾系統處理後之產物，其鈣/鎂離子比例可達 0.6:1，亦較深層海水原水之 0.3:1 為高，可知此系統具有提升產物中鈣鎂比例的能力。此方法提供了一個有用的過濾路徑與操作條件，可有效的去除深層海水中的硫酸根離子並維持適當的礦物質含量，可用以製備脫硫之高鈣鎂比之礦物質水。

21. Removing sulfate ions from deep seawater through a multi-step membrane filtration process

°Yu-Fong Huang , Cheng-Shing Lo, Chang-Hao Cheng and Ping-Yi Huang
(Stone and Resource Industry R&D Center, Taiwan)

I. Introduction

Deep seawater (DSW) has attracted substantial attention because of its low temperature, cleanliness, contents, and potential applications in food, agriculture, and renewable energy. Researchers have recently focused on separating and concentrating mineral contents from DSW to extend its applications; however, sulfate ion removal must be considered prior to the concentration process. The failure to address sulfate ions reduces volumetric capacity and causes premature equipment failure; precipitate formation (e.g., calcium sulfate) also decreases the mineral content of water. This study involved developing an efficient nanofiltration (NF) process to remove sulfate ions and preserve desirable minerals in DSW.

II. Experimental

The proposed system comprised 2 NF units. The first NF unit, NF1, contained a DSW tank, a pre-treatment filter to remove particles from DSW, a high pressure plunger pump, a NF tubular membrane module, and a buffer tank. A stream (concentrate or permeate) was selected as the feeding source for the second NF process (Figure 1). The second NF unit, NF2, contained a buffer tank, high pressure pump, and an NF tubular membrane module. ICP-OES (Optima 2,100 DV, PerkinElmer Instruments, Columbia, MD, USA) was conducted to determine the mineral concentrations in waters.

III. Results and Discussion

Filtration route and operational pressure played critical roles in mineral contents in the product. The analyses indicated that the C + P filtration route attained a higher (98.8%) sulfate ion rejection rate than that in the P + C route (62.2%), indicating that P + C route is unsuitable for DSW sulfate removal. Thus, the P + C filtration route was applied for DSW in this study. Subsequently, the operation pressure was increased to promote mineral retention in products. Notably, when the operational pressure increased to 150 psi, retention of calcium and magnesium ions reached 72.5% and 34%, respectively. Moreover, the sulfate ion rejection remained approximately at 97%. These findings indicate there is an effective filtration process that rejects sulfate ions from DSW and retains the minerals in the products.

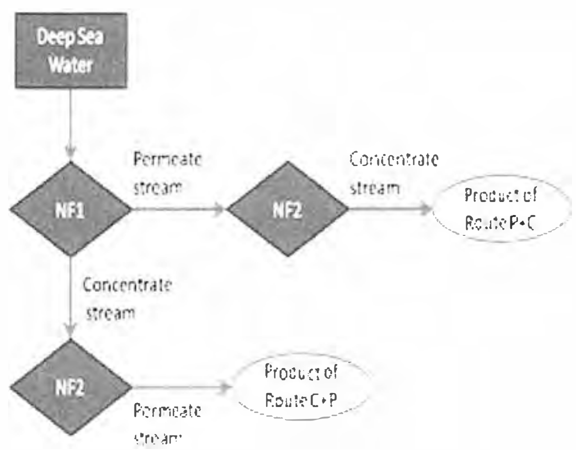

Figure 1. Schematic diagram of NF filtration processes.

一般講演５
〈利活用システム関連他２〉
Session 5:　Resource Applications　2

座長　　李　士畦

（工業技術研究院　特別補佐）

Chairman: Shih-Chi Lee

(Center Executive Assistant, Industrial Technology Research Institute)

22. Hardness Water Production From Deep Seawater By RO/NF/ED Linking Process

°Deok-Soo Moon, Kwang Soo Kim, Ho Ji, Mi Yeon Choi, Hyun Ji Jung and Hyeon-Ju Kim
(Deep Ocean Water Application Center, Korea Institute of Ocean Science & Technology)

Reverse osmosis (RO) membranes can separate dissolved materials and freshwater from seawater (deep seawater). The desalinated water passing through the second RO membrane was completely removed of its dissolved substances of more than 99.9%, resulting to its hardness concentration being 1 mg/L or less, and its chloride concentration 2.3 mg/L. Since the nano-filtration (NF) membrane pore size is 10^{-9} m, 50% of magnesium and calcium ions cannot pass through the NF membrane, while more than 95% of sodium and chloride ions can pass through the NF membrane. NF membrane could separate salt components like sodium and chloride ions from hardness ingredients like magnesium and calcium ions, but their separation was not absolute. Electric dialysis (ED) membrane system can separate single charged ions (like sodium and chloride ions) and double charged ions (like magnesium and calcium ions) independently depending on their electrical conductivity. At the electrical conductivity of more than 20mS/cm, hardness components (like magnesium and calcium ions) were not removed. On the other hand salt ingredients like sodium and chloride ions were removed continuously.

Thus, we were able to concentrate hardness components (like magnesium and calcium ions) using the NF membrane which also could separate salts ingredients from the hardness concentration water using ED membrane system. Finally, we were able to produce a highly concentrated mineral water with its chloride ions removed with hardness concentration of 12,600 mg/L and chloride concentration of 2,446 mg/L. By diluting 10 times with the secondary desalinated water obtained by RO, this highly concentrated mineral water could produce high mineral water suitable for drinking water standards with chloride concentration of 244 mg/L and hardness concentration of 1,260 mg/L.

Using the linked process with RO/NF/ED, hardness components like magnesium and calcium ions could be concentrated, while at the same time removing salt ingredients like chloride and sodium ions without heating. Thus, using only RO, NF and ED membranes without heating seawater can possibly produce drinking water containing high hardness suitable for drinking water standards, while reducing the energy required for evaporation.

23. Design and experiment of 20kW Ocean Thermal Energy Conversion pilot plant

°Ho-Saeng Lee, Sang-Wong Cha, Young-Seok Kim, Ji-Hoon Son, Hyeon-Ju Kim
(Deep Ocean Water Application Center, Korea Institute of Ocean Science & Technology)

The potential capacity of ocean thermal energy conversion is known to be 1.1 trillion kW or 100 times bigger than the total energy demand of the world as of 2000. The appropriate locations for a large-scale commercial plant are on the ocean along the equator and in the subtropical regions between the 20° north and south latitudes, where a higher energy density can be obtained from the ocean temperature difference. If the application of various unused energy sources (such as solar, ground, wastewater, thermal, and ocean thermal energy) in higher-latitude regions is diversified, the commercialization of such energy sources is considered possible, to cover small- and medium-sized demands for power. Ocean thermal energy conversion (OTEC) is regarded as a less variable and sustainable new energy source, but its commercialization requires phased demonstrations and efforts to reduce the power generation cost through a business strategy. In this regard, countries such as the U.S. and Japan have been promoting various developments and researches in this field, and its feasibility has been presented in analytical studies on the initial investment required and the power generation cost. To experiment with 20kW OTEC, the closed-cycle type of OTEC was designed and manufactured. R32 (Difluoromethane, CH_2F_2) was used as the working fluid and the temperatures of heat source and heat sink were 26°C and 5°C, respectively. A semi-welded type heat exchanger was applied for the evaporator and condenser and the cycle was designed for the gross power of 20kW. The semi-welded type heat exchanger has strong points such as easy maintenance of the gasket type heat exchanger and infrequent leakage of welded type heat exchanger. In the plate arrangement of the semi-welded type heat exchanger, one channel for working fluid is welded, and another channel is gasketed.

In this paper, various performance evaluation and experiment were carried out in constructing subminiature pilot plant of the OTEC and compared with the results of cycle analysis.

ACKNOWLEDGEMENTS

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24. Establishment of Blue Infrastructure using Seawater Resource in Coastal Areas

Hyeon-Ju Kim, Ho-Saeng Lee, Deok-Su Moon and Wee-Yeong Oh
(Deep Ocean Water Application Center, Korea Institute of Ocean Science & Technology, Goseong-gun, Korea)

For most resident population and industrial complexes in coastal areas, the demand for food, energy, and water is growing fast. In Korea, the total domestic energy consumption is 271.4Mtoe, out of which 35% of the total consumption, 96.3Mtoe, is estimated to be spent for air conditioning and heating in coastal areas. Desalination for agriculture and industrial factories needs plenty of energy, therefore, water shortage often directly relates to energy issue. The ocean water, 97% of the entire water supply in the world, has enormous dissolved substances and energy as potential heat source. Thus, people in the coasts can get resource and energy from the ocean that can easily be a way to overcome fossil fuel dependence. Twenty-seven percent of the Korean population lives in cities along the coast, and 71% of industrial complexes are near the coasts. About 10% of the population is estimated to live within 4 km from the coasts who can use ocean thermal energy for air conditioning. In addition to this resident population, there are only eight deep ocean water intake facilities, but power plants, industrial factories, fisheries experiment stations, and onshore fish farms require at least 160 million tons of deep ocean water (DOW) a day. Intake and discharge of DOW for these facilities can be a great source for ocean thermal energy conversion (OTEC). For instance, with a thousand of 1000RT ocean water source heat pumps, the national renewable energy goal can be accomplished by supplying 900,000 toe from ocean energy. Electricity required for operating the system, 300,000 toe, could be obtained from multi-heat OTEC systems that can be a start of clean production and life in a non-oil economy with no fossil fuel consumption. Therefore, green infrastructure using blue resource can complete “Blue Infra” for the future. A national project to supply test beds, step by step ways for thermal energy utilization is in the process. For the first step, 250 sets of 1000 RT heat pumps, and for the second step, an additional 1000 sets of 1000 RT heat pumps would be distributed. With this project, 222,500 toe of renewable energy production and 454,500 ton of carbon dioxide reduction can be accomplished in the first step, then 890,000 toe of renewable energy production and 1,818,000 ton of carbon dioxide reduction will be accomplished in the second step.

ACKNOWLEDGEMENTS

This work was financially supported by the National R&D project of “Development of Energy Utilization of Deep Ocean Water" supported by the Korean Ministry of Oceans and Fisheries.

25. The effect of feed temperature on permeate flux during membrane separation

°K. S. Kim, D. S. Moon, H. J. Kim, S. W. Lee, H. Ji, H. J. Jung, H. J. Won

(Deep Ocean Water Application Center, Korea Institute of Ocean Science & Technology)

Feed temperature has an effect on the desalination performance of seawater by membrane separation. When the permeate flux tends to increase using the waste heat, it is necessary to precisely analyze the effect of feed temperature on membrane performance. The experiments were carried out to investigate the performance of membranes by varying the seawater temperature from 10℃ to 60℃. The increase of permeate flux with increase of feed temperature was interpreted as the change of water viscosity and the membrane itself. While the increase of permeate flux could be predicted by the viscosity change in case of nano-filtration membrane, there exists a 30% difference between the experimental data and the prediction by the viscosity change in the case of reverse osmosis (RO) membrane, which seems to be due to an 8% decrease of the pore size in 60℃ caused by the contraction of membrane with the increase of temperature. Therefore, the desalination of seawater should be carried out within the range that the elevation of temperature does not cause the alteration of the membrane itself even for the purpose of increasing the permeate flux.

ポスター・セッション

Poster Presentation

P1. Distribution of water masses and variation of nutrients in the coastal areas of Gangwon Province of the Korean East Sea from 2008 to 2012

°Mi-Yun Choi, Deok-Su Moon, Hyeon-Ji Jung, Young-Suk Kim and Hyeon-Ju Kim
(Deep Ocean Water Application Center, Korea Institute of Ocean Science & Technology)

The distribution of water masses and variation of nutrients according to the change of seasons and locations were investigated 4 times per year in the coastal areas of Gangwon province of the Korean East Sea from 2008 to 2012. The surveys of the physico-chemical parameters (temperature and salinity) and nutrients (NO_2-N, NO_3-N, NH_4-N, PO_4-P, and SiO_2-Si) were carried out at 6 locations (Goseong, Sokcho, Yangyang, Gangneung, Donghae and Samcheok). The water masses included in the study area were divided into 4 groups; 1) Tsushima Surface Water (TSW), 2) Tsushima Middle Water (TMW), 3) North Korean Cold Water (NKCW), and 4) East Sea Proper Water (ESPW). The distribution of water masses was affected by the change of season. In February, surface water was derived from the TMW. The TSW was not observed in May, but only observed in August. In November, as the influence of the TSW weakened, that of the NKCW strengthened. The average concentration of nitrate for 4 years at the surface was 12.322 μM and at the bottom was 15.700 μM. The average concentration of phosphate for 4 years at the surface was 1.086 μM, and at the bottom was 1.331 μM. Silicate for 4 years at the surface was 20.706 μM, and at the bottom was 1.331 μM. The annual average concentration of nutrients at the surface showed the highest concentration in 2009. The annual average concentration of nutrients at the bottom increased sharply starting in 2010, and showed the highest concentration in 2011. The seasonal average concentration of nutrients at the surface showed the highest concentration in February, and gradually decreased thereafter. On the other hand, the seasonal average concentration of nutrients at the bottom increased starting in February, and showed the highest concentration in August. The average surface concentrations of nutrients at various locations showed a similar concentration, but showed the lowest values at Samcheok.

P2. 発芽体集塊化法による深層水コンブ生産の事業化

○平岡雅規[1]、蜂谷 潤[2] ([1]高知大学、[2]一般社団法人うみ路)

１．はじめに

高知県室戸市では海洋深層水を使った食用青海苔・スジアオノリ生産施設が 2004 年から稼働しており、年間の生産量は乾重量 2t 以上で 2009 年に黒字化している。2012 年には当初計画されていた年間生産量 3t にほぼ到達した。この施設では高効率海藻生産を実現するために、我々が開発した浮遊式で海藻を高密度タンク栽培できる胞子集塊化法(胞子および発芽体の集塊化による海藻養殖法:特許第 3828359 号)とシステムへの投入エネルギーを節約できる多段式タンクシステムが採用されている。これらの技術は、胞子を造って増殖する海藻に広く応用できる。スジアオノリの生産事業では一定の成果が得られたので、次の事業化を目指す海藻としてコンブ(マコンブ)を選定した。通常、食用コンブは数 m の長さに生長した成体を加工したものが市場で販売されているが、胞子集塊化法を使ったタンク栽培では長さ 10-20cm 以下の小さい幼体が集塊化した新しい形状のコンブ(図 1)が大量に生産できる。幼体コンブ生産の事業化に向けた実証試験を実施した。

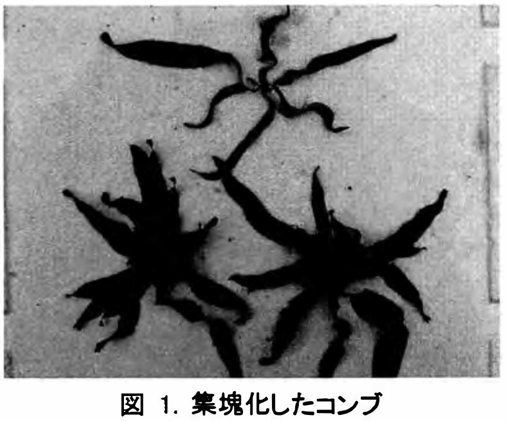

図 1. 集塊化したコンブ

２．材料及び方法

成熟したコンブを宮城県で採取した。採取したコンブから遊走胞子を分離して培養し、微小世代である雌雄配偶体の単藻株を確立した。実験室で雌雄配偶体を増殖させて精子と卵の形成を誘導し、受精させてシャーレ内で発芽体を高密度で発生させた。培養過程で発芽体は互いに連結して発芽体集塊を形成した。これら発芽体集塊をシャーレ底面から取り外し、通気して培養容器内で浮遊させながら発芽体の長さが 2-3mm 以上になるまで培養した。生長した発芽体集塊を種苗として、屋外に設置した 1t 円形水槽に室戸市で取水されている海洋深層水を連続注水しながら栽培し、2 日毎に湿重量を計測した。この栽培試験は 2012 年 8 月から 2013 年 2 月に繰り返し実施された。

３．結果及び考察

屋外タンクでのコンブ栽培初期の指数生長期にあたる日間生長率は 18%から 39%と変動し、平均で 28±7.4% (±標準偏差, n=7) であった。また、タンク容量の 1/1000 を超えると生長率の低下を示した。これらの結果から 6 日間で 0.25kg が 1kg に生長する条件で多段式タンクシステムを構築した。0.04t、0.16t、0.6t、2.5t、10t の 5 段階からなるタンクシリーズを組むと、6 日毎に湿重量 10kg 生産できると試算された(図 2)。

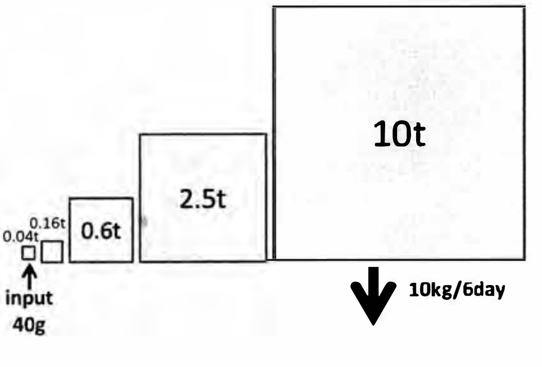

図 2. コンブ生産用多段タンク例

P2. Kombu (*Saccharina japonica*) commercial production using deep seawater and germling cluster method

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1. Introduction

A commercial-scale plant for the production of edible green laver, *Ulva prolifera* using deep seawater (DSW) was established in Muroto, Kochi Prefecture, Japan in 2004. The plant produces more than 2t-dry weight per year and has been operating with a positive balanced budget financing since 2009. Actual laver production in 2012 reached approximately 3t-dry weight that is the maximum target for the plant. To realize intensive biomass production, this plant uses a new "germling cluster method" from our invention (Japan patent number 3828359) for intensive tank culture of seaweeds in a free-floating form and multistep tank series for saving energy input into the system. These methods can be applied to a wide range of seaweed species reproducing by means of spores. As the commercial production of *U. prolifera* has worked successfully, we selected kelp, *Saccharina japonica* (called "kombu" in Japan) for the next commercial-scale production. Generally, the food products processed from well-developed adult plants several meters long are sold in the market. However, using our culture technique, juvenile kombu clusters of less than 10-20 cm in length (Figure 1) can be produced in large quantities as the new products. We carried out a test examination for the commercial-scale production of the juvenile kombu plants.

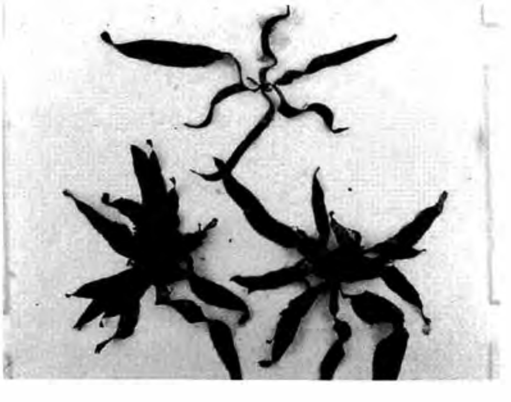

Figure 1. Juvenile kombu clusters

2. Material and Methods

Mature kombu was collected in Miyagi Prefecture, Japan. Swimming spores were isolated and cultured to establish male and female micro-gametophytes as unialgal strains. The formation of sperm and eggs was induced on the male and female gametophytes and fertilized in the laboratory. By culturing fertilized eggs at a high density in a glass dish, the dense germinating bodies spontaneously connected with each other and formed clusters. The clusters composed of several germlings were removed from the bottom of the dish and cultured in a glass flask with aeration as free-floating forms until the length of the germlings became more than 2-3 mm. The developed germling clusters were transplanted to a 1t outdoor cylindrical tank continuously supplied with DSW pumped up in Muroto and the fresh mass of the clusters was measured every 2 d. The culture experiments were repeatedly conducted from August, 2012 to February, 2013.

3. Results and Discussion

Daily growth rate of kombu in the initial stage of the exponential growth in the outdoor tank fluctuated from 18% to 39% and was 28±7.4% on the average (±standard deviation, n=7). In addition, the growth rate decreased when the biomass in the tank surpassed 1/1000 of the tank volume. From these results, we assumed a multistep tank series on the presumption that a kombu biomass of 0.25 kg can grow up to 1 kg after 3 d. In a tank series consisting of five steps of 0.04t, 0.16t, 0.6t, 2.5t and 10t, 10 kg of kombu biomass is calculated to be harvested every 6 d (Figure 2).

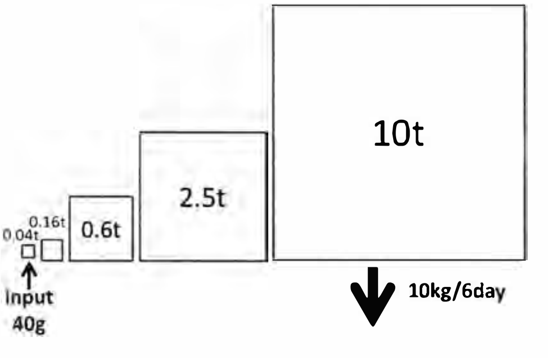

Figure 2. A multistep tank series for kombu production

P3. 陸地及海洋微生物應用於永續能源開發之評估研究

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I. 前言

針對利用陸地及海洋微生物燃料電池(MFCs)於同時生物產電及脫色(SBG&RD)之能源再利用之研究, Sun *et al*., Li *et al*., Cao *et al*.及 Chen *et al*. 首度提及以混菌及純菌 MFC 系統來結合脫色及生物產電, 並得到相當顯著之產電效能(>95%脫色率及 500-600 mW m^{-2} 之最大功率密度)。再者, 新近應用於海水介質電子轉移之海洋生物膜研究, 更顯示於含鹽水介質中進行生物產電確實具有正面實用性。但是 SBG&RD 之操作可行性及穩定性之確定問題仍不明朗, 仍有待後續之研究澄清, 以利於廣泛應用。先前研究更提及脫色微生物(例如: *Proteus hauseri*、*Klebsiella pneumoniae* 等)於 SBG&RD 操作下之生物特性, 此研究則首度嘗試以更系統之追蹤研究來說明於含鹽水介質中, 經長時間連續之馴養作用產生具電化學活性之微生物以進行 SBG&RD, 確實具有其正面之可能性。但是由於菌相生態產生明顯之生態消長作用, 本研究建議對於 MFC 中之海洋菌相生態之演化過程, 應深入解讀, 以利於後續之永續能源開發應用。

II. 材料與方法

單槽式無質子交換膜型之空氣陰極 MFC 建構於以 PMMA 壓克力材質建構之圓柱體管柱(電池尺寸 ID=54 mm, L=95 mm, 操作體積約為 220 mL)。其中不含防水或觸媒之多孔性碳布(CeTech™; 投影面積約$\pi \times 2.7^2$=22.9 cm^2), 以一面用做陽極電極。與陽極面積約略相等大小之空氣陰極則是以 PTFE 擴散層(CeTech™)來建構空氣接觸面。研究所用之產電海洋微生物- *Exiguobacterium acetylicum* NIU-K2 則是由宜蘭太平洋岸蘭陽溪出海口生態保護區域之污泥中, 以含鹽水之 LB 培養基進行連續優勢馴養篩選, 並經由 16S rRNA 親緣樹分析所得到。本 MFC 研究中之殖種細菌培養則是以 LB (Luria-Bertani)培養基(單位: g L^{-1}) (tryptone 10 g L^{-1}, 酵母萃取物 5 g L^{-1}, 氯化鈉 10 g L^{-1})。為馴養含海水 MFCs 以進行研究, 富鹽 LB 培養基用以添加入 MFC 中分別含有 tryptone 10.0、酵母萃取物 5 g L^{-1} 及氯化鈉 60.0 g L^{-1}。首先以勾菌環勾取 LB 瓊脂平板(即在添加 20 g L^{-1} Bacto agar 之 LB 固體培養基平板)於四分劃線所得之單一菌落, 以含 200 mg L^{-1} 反應藍 160 (RBu160)偶氮染料之 50 mL LB 培養液進行 30°C, 125 rpm 之過夜(O/N)前培養。一旦脫色至殘留染料濃度降至 5%以下, 培養菌液即行收獲, 進行 2400 X *g* 10 分鐘高速離心。後去除上清液, 以去離子之殺菌蒸餾水進行沖洗後, 再度高速離心後, 去除上層液(重覆兩次此步驟), 以收獲菌泥 5 mL, 以進行殖種於 MFC 中。

III. 結果與討論

先前研究發現經過以兩天之周期來添加染料及 LB 培養基以進行連續馴化刺激, 約一個月可達到穩定之陽極生物膜形成及穩定產電之結果。事實上, 先前研究室研究成果亦指出固定化生物膜系統約經 200-400 小時(即 9-18 天), 由於吸脫附可能已達到動態平衡, 因此可在連續操作下, 達到懸浮菌體濃度持平之結果。因此為了評估觀察長期產電性能之穩定性, 含鹽 MFC 經過周期式之連續 SBG&RD 馴化培養下, 進行操作約 340 天後, 已可假設生物產電性能應已達到動態平衡。然而, 本研究結果更發現經約經一年之長期 MFC 操作後, 產電已漸次偏離原先之產電穩定狀態。此點更代表此 NIU-K4 殖種之 MFC 產電性能已逐漸因為被其他外來開放之污染菌所取代而式微, 造成達到另一新穩定產電之平衡點。再者以分子生物學之證據指出, 去活梯度膠體電泳(DGGE)之圖譜更說明經過一年 MFC 操作後, 此系統已被耐鹽之優勢菌相生態所取代(例如: *Vagococcus lutrae*, *Rhizobium rosettiformans*, *Sporanaerobacter acetigenes*, *Pseudomonas plecoglossicida*)。但是此產電系統之菌相生態族群動態消長情形, 究竟是以何種途徑或是方式(例如:合作互利共存或是競爭消長取代)來達到, 仍有待更進一步之深入探討, 以利於工業化應用之評估。

IV. 結論

利用含本土 *Ex. acetylicum* NIU-K4 殖種之海水介質 MFC 之同時生物產電及脫色於長期處理操作是具有操作可行性。添加反應藍 160 偶氮染料進行電子轉移之馴養, 以刺激具電化學活性菌體之產電活性產生, 是一可行之操作策略。然而, 經過超過一年之長期操作後, 產電活性雖然存在, 但是原有殖種菌 NIU-K4 卻已式微。此點說明產電菌相生態演化之深入調查研究, 方是決定海水 MFC 穩定處理之關鍵因素。

誌謝

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P3. Exploring characteristics of land-based and marine microbes for sustainable energy applications

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I. Introduction

Regarding simultaneous bioelectricity generation and reductive decolorization (SBG&RD) using land-based and marine microbial fuel cells (MFCs), Sun *et al.*, Li *et al.*, Cao *et al.* and Chen *et al.* reported pioneer findings to demonstrate a promising feasibility of dye decolorization (ca. >95% decolorization) coupled with bioelectricity generation (ca. max. power density~ 500-600 mW m^{-2}) using mixed or pure culture-bearing MFC systems. In addition, marine biofilms used for electron transfer in seawater revealed promising practicability for bioelectricity generation in salty media. However, the feasibility of SBG&RD in salty environments was less clear and possible applications are still being explored. As prior studies explored microbial characteristics of SBG&RD using pure biodecolorizers (e.g., *Proteus hauseri*, *Klebsiella pneumoniae*), this pioneering study systematically indicated that simultaneous reductive decolorization and power generation of salt-tolerant microbes in salty media was technically-feasible and electrochemically-active after serial acclimatization for long-term stable operation. This study suggested that ecological evolution of marine microbial population in MFCs should be investigated prior to possible sustainable energy applications.

II. Materials and Methods

Membrane-free air cathode single-chambers for schematic setup of MFC were constructed in cylindrical tubes made by polymethyl methacrylate (PMMA) (cell sizing ID=54 mm, L=95 mm) with the operating volume of 220 mL. Porous carbon cloth (CeTech^{TM}) (without waterproofing or catalyst) with a projected area of ca. 22.9 cm^2 (i.e., $\pi \times 2.7^2$) on one side was used as anode electrodes. The air cathode was almost identical to the anode in size and consisted of a polytetrafluorethylene (PTFE) diffusion layer (CeTech^{TM}) on the air-facing side.

The marine microbe- *Exiguobacterium acetylicum* NIU-K2 primarily isolated from Langyang River Basin near Pacific-Ocean coast in I-Lan, Taiwan through serial acclimatization in progressively salty LB broth cultures and identified via 16S rRNA phylogenetic tree analysis was used for study. Bacterial cultures were carried out as described elsewhere. Culture media in MFCs used in this study (unit: g L^{-1}) were LB broth medium (tryptone 10, yeast extract 5, sodium chloride 10), 0.5 x LB (tryptone 5.0, yeast extract 2.5, sodium chloride 10). To acclimatize salty MFCs for study, the salty LB medium in MFC (simply denoted as salty MFC) as stated afterwards contained tryptone 10.0, yeast extract 5.0 and sodium chloride 60.0 g L^{-1}. A loopful of strain seed taken from an isolated colony on a LB-streak plate (i.e., LB medium supplemented with Bacto agar 20 g L^{-1}) was precultured in 50 mL LB broth laden with 200 mg L^{-1} reactive blue 160 (RBu160) using 250 mL Erlenmeyer flask for overnight (O/N) at 30°C, 125 rpm. Once residual dye concentration of the O/N culture was completely decolorized (ca. < 5%), cultured cells were then harvested and collected after centrifugation at 2400 X *g* for 10 min. After cell-free supernatant was discarded, the biomass was rinsed and mixed with DI/DD (deionized/distilled) water. Rinse-and-mix procedures were repeated twice to harvest residue-free 2x rinsed biomass and then 2x rinsed biomass was concentrated with DI/DD water in 5 mL for inoculation to MFC.

III. Results and Discussion

After stable power production in salty MFC had been achieved 340 d after inoculation, repeated tests of SBG&RD were conducted. According to Chen *et al.*, cell population immobilized onto anodic biofilm could be stabilized at ca. 30 d after repeated acclimatization. In addition, prior study also revealed that nearly time-invariant suspended cell concentration could be stably achieved at *ca.* 200-400 h (*ca.* 9-18 d) after serial acclimatization, since adsorbed and desorbed cell concentration was in equilibrium even under a selection pressure of washout present in continuous cultures. Thus, stable cell population in MFC stated herein could be postulated to be in equilibrium after 340 d of operation. However, after *ca.* 1 year long-term MFC operation the species profiles in this NIU-K4 seeded MFC might have gradually evolved to another steady and stable ecology in equilibrium. To reveal the mysteries behind the ecological changes, denaturing gradient gel electrophoresis (DGGE) profiles were analyzed. As shown in DGGE profiles, some salt-tolerant species were apparently evolved in MFC after *ca.* 1 year long-term acclimatization (e.g., *Vagococcus lutrae*, *Rhizobium rosettiformans*, *Sporanaerobacter acetigenes*, *Pseudomonas plecoglossicida*). However, how and why population dynamics with combined interactions among the strain NIU-K4 and other introduced foreign species evolved to a steady state in MFC still remained to be investigated for further studies.

Acknowledgment

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P4. 解析海洋深層水微生物菌種組成及對病源真菌(*Fusarium oxysporum*)具抑制作用之菌種篩選

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林怡汝、黃秉益(石資中心)、黃介辰(國立中興大學)

1. 前言：

我國四面環海如何有效利用豐富的海洋資源以促進產業發展是一項重要的課題。海洋微生物為適應高壓高鹽、低光照低溫與寡營養的環境，因此演化出與陸地微生物相異之基因功能與生化代謝途徑，可生產特殊結構與功能的天然化合物與酵素，極具開發潛力。近年來國際期刊報導從海洋微生物發現之化合物近三百種，另發現許多可分泌抑制真菌及抗藥性菌株生長之菌種，不同於既知之陸地微生物。

本研究自等鞭金藻海洋深層水培養基及海洋深層水過濾濾紙中解析菌種組成與菌株功能，並進一步探討分離菌株對真菌 *Fusarium oxysporum* 生長抑制效果。尖鐮胞菌(*Fusarium oxysporum*)屬於土傳性病原菌，可感染各種苗木、蔬菜、瓜果、花卉發生植物萎凋病，如馬拉巴栗、香蕉、西瓜、辣椒、百合等作物造成嚴重損失。海洋微生物除可能擁有特殊抑菌效果之化合物外並兼具耐高鹽與極端環境特性是作為開發生物農藥的理想菌株來源。

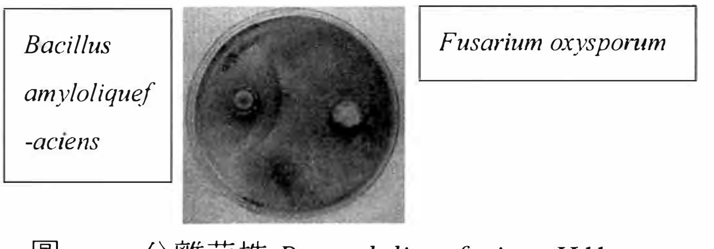
2. 實驗方法：

本研究為模擬海洋微生物在高鹽環境，在每公升培養基加入 2% NaCl 高鹽濃度及微量元素 $FeCl_3$、$MgCl_2$、$CaCl_2$、KCl、$NaHCO_3$、H_3BO_3、Na_2O_3Si、NH_4NO_3、Na_2HPO_4 與 0.1% yeast extract、0.5% peptone 置於 25℃恆溫培養箱中進行海洋微生物培養與菌種篩選。將海洋深層水過濾膜與等鞭金藻海洋深層水培養基以序列稀釋成 10^{-1}~10^{-8} 做純菌分離培養。並將得到之海洋深層水分離菌種萃取 DNA 以 16s rDNA PCR 擴增序列後進行基因定序。將培養的海洋分離菌液吸取 50 ul，與預培養七天之尖鐮胞菌(*Fusarium oxysporum*)鑽取直徑 0.5cm 放置 PDA(Potato dextrose agar)培養基，以 25℃恆溫培養約七天試驗拮抗效果。

3. 結果與討論：

海洋深層水過濾膜之菌種分析結果顯示有 *Bacillus aquimaris*、*Lysinibacillus fusif-ormmmis*、*Bacillus cereus*、*Bacillus subtilis*、*Bacillus aquimaris*、*Bacillus amyloliquefaciens*、*Bacillus aerophilus* 等微生物。等鞭金藻海洋深層水培養基之菌種分析結果顯示 *Alteromonas sp.*、*Vibrio sp.*、*Halomonas sp.*、*Bacillus sp.*、*Jeotgali-bacillus* sp.等微生物。將兩者來源之海洋微生物分離株所測試之拮抗實驗結果發現，來自海洋深層水過濾膜之分離株 *Bacillus amyloliquefaciens* H11 對真菌有良好的拮抗效果(圖一)。

此分離菌株 *B. amyloliquefaciens H11* 曾被報導發現是水稻及菸草之內共生菌，未來可進一步深入探討分泌之抑菌化合物結構與酵素功能。



圖一、 分離菌株 *B. amyloliquefaciens H11* 對真菌 *Fusarium oxysporum* 抑制效果

P4. Profile of a deep sea water microbial community and screening of bacterial strain showing antagonistic activity against the plant pathogen, *Fusarium oxysporum*

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I. Introduction

Taiwan is surrounded by seas on all sides. The development of a marine resources industry becomes an important issue. Marine bacteria growing in high salinity, high-pressure, low temperature and dilute nutrients may cause the evolution of special biochemical and enzymes functions different from those of terrestrial origins. According to scientific reports, 273 different chemical structures have been found in marine bacteria and many different bacterial strains can suppress fungal growth or drug-resistance.

This study isolated bacteria from *Isochrysis galbana* cultured in deep sea water (DSW) medium and DSW filtration membranes and tested for antagonistic activity against plant pathogen. *Fusarium oxysporum* can induce the nursery stock, vegetables, fruits, flowers withering, like the banana, malabar chestnut, watermelon, pepper, lily. Some marine bacteria can suppress specific fungi and their high salinity tolerance is a good resource for exploring bio-pesticides.

II. Materials and Methods

This study simulated a high salinity environment, where 2% NaCl was added as well as the other trace elements such as $FeCl_3$, $MgCl_2$, $CaCl_2$, KCl, $NaHCO_3$, $H3BO_3$, Na_2O_3Si, NH_4NO_3, Na_2HPO_4 and 0.1% of yeast extract, 0.5% of peptone placed in 25 ℃. Amplification of 16s rDNA gene for sequencing was done after extracting the DNA of isolated strains. For fungus antagonistic activity, 50 ul of cultured isolate and drilled diameter 0.5 cm pre-cultured for 7 d. *F. oxysporum* was placed in PDA (Potato dextrose agar) medium in 25 ℃ and incubated for 7 d.d

III. Results and Discussion

The isolate strand form DSW filtration membranes showed *Lysinibacillus fusiformis*、*Bacillus cereus*、*Bacillus subtilis*、*Bacillus aquimaris*、*Bacillus amyloliquefaciens*、 and *Bacillus aerophilus*. *Isochrysis galbana* grown in DSW culture medium contained *Alteromonas sp.*、*Vibrio sp.*、*Halomonas sp.*、*Bacillus sp.* and *Jeotgalibacillus* sp.. The result of isolated *Bacillus amyloliquefaciens* H11 showed a good effect for suppressing *F. oxysporum*.

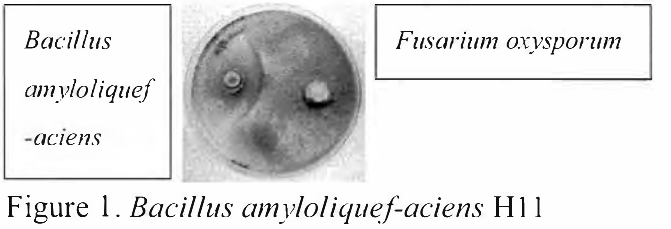

Figure 1. *Bacillus amyloliquef-aciens* H11 antagonistic activity against *Fusarium oxysporum*

P5. 建構一套兼具低成本及以深層海水為培養基之微藻葉黃素生產系統

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一、前言

葉黃素為一強抗氧化成份，且對抗癌、抗老化、保護皮膚、預防慢性病、老年眼睛黃斑退化症、阻抑紫外線等皆具有顯著的功效，因此近年來常利用於保健食品之原料。目前金盞花是主要商業化葉黃素之原料來源；然而，以金盞花為來源恐有重金屬污染之虞、且有勞力密集、較長的生長週期以及較低的葉黃素含產等缺點。有鑑於此，近來微藻被視為重要的葉黃素新興來源之一，其優點包含：(a)藻體可全部利用(毋需移除植物之花瓣)、(b)微藻生長速度快可得較大之葉黃素產率、(c)相較於金盞花等植物，微藻培養可得較高含量之葉黃素。

二、實驗方法

藻種篩選

本研究使用之藻株為 *Scenedesmus obliquus* CY10，於台灣中南部水域所篩選之本土藻種。

藻體培養與分析方法

本研究利用 TL5 日光燈管以 150 μmol/m^2/s 光照強度於 1L 之光生物反應器中進行全日光照培養，溫度為 28℃,pH 值控制為 6.0，攪拌速率為 300rpm，並持續地以 2.5% 之 CO_2 氣體當做培養系統之碳源曝氣。每日進行適量藻液取樣，以分光光度儀(UV-VIS)於吸收波長 680 nm 下測定其吸光值，再將之換算成藻體細胞乾重以得藻體濃度。

光照強度測定

本研究以光照度計(LI-250) 搭配 LI-200SA 感測器來量測光源強度，其單位為 mol/m^2/s，藉以監控制光照強度

三、結果與討論

本研究首先由南台灣海域篩選出本土柵藻藻株 (*Scenedesmus obliquus* CY10)，發現其富含葉黃素 (lutein)，且利用 10-20%的深層海水進行培養時，其葉黃素含量可達 3.5 mg/g DCW。將微藻培養系統放大至 50 公升，並相同以 10-20% 的深層海水當作培養基時，其葉黃素含量仍可維持在 4.06 mg/g DCW。本研究並進一步開發最適化葉黃素萃取程序，證實添加適量的深層海水確實可有效提升微藻生長與葉黃素之產量。

關鍵字：深層海水、柵藻、葉黃素

P5. Enhancing microalgae-based lutein production with *Scenedesmus obliquus* CY10 using deep seawater as the cultivation medium

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I. Introduction

Lutein is one of the xanthophyllic families containing hydroxyl or carbonyl groups that enhance their solubility in animal tissues. Due to its ability to prevent or ameliorate cardiovascular diseases, some types of cancer, and degenerative human diseases, lutein is frequently used as a food additive.

At present, marigold petals are used for commercial production of lutein. However, this source presents some disadvantages, like the possibility of heavy-metal pollution, labor-intensive extraction process, long growth period, and the extremely low lutein content of the plants (as low as 0.03%). In recent years, microalgae have been attracting attention as a promising alternative to produce lutein. The advantages of using microalgae as the lutein source include: (a) excluding the need to remove petals from plants the whole biomass of the microalgae can be processed, (b) producing microalgae at a high growth rate thereby giving a high lutein productivity and (c) yielding relatively high lutein content at high biomass productivity compared to marigold and other terrestrial plants.

Deep seawater (DSW) generally refers to seawater from a depth of more than 200 m. It is known to contain some specific trace elements and nutrients that could be beneficial for the growth of microalgae or for stimulating the production of specific components in the microalgae.

II. Materials and Methods

Isolation of microalgae

The microalga used in this study was *Scenedesmus obliquus* CY10, isolated from freshwater located in southern Taiwan.

Microalgal culture

The *S. obliquus* CY10 strain was grown under a light intensity of approximately 150 μmol/m^2/s (illuminated by TL5). The 1-liter photo-bioreactor (PBR) was operated at 28 ◦C, pH 6.0, and an agitation rate of 300 rpm. Serving as the sole carbon source, 2.5% CO_2 was fed into the culture continuously. The OD680 values were converted to biomass concentration via proper calibration between OD680 and dry cell weight (i.e., 1.0 OD680 approximately equals 300 mg DCW/l).

III. Results and Discussion

In the present study, microalgae have emerged as a promising source for lutein production. In this study, an indigenous microalga *S. obliquus* CY10 isolated from southern Taiwan has a high lutein content, and was thus examined for its potential as a commercially viable lutein source.. When *S. obliquus* CY10 was cultivated in medium amended with 10-20% DSW, its lutein content could reach nearly 3.5 mg/g DCW. Next, the microalgal cultivation system was successfully scaled-up to 50 liter PBR with the addition of 10-20% DSW. The results showed that the lutein content is still maintained at 4.06 mg/g DCW. The optimal conditions for lutein extraction and isolation were also established. This work showed that addition of an appropriate amount of DSW into culture medium could significantly enhance the microalgal growth and lutein production.

P6. 利用深層海水畜養長莖葡萄蕨藻對其萃取物之免疫細胞活性的影響

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1. 前言：

深層海水是指有光層以下的海水，陽光無法進入，具低溫、富礦物質及營養、清澈乾淨、病原菌稀少等特性，可以應用在食品、養殖、農業等產業。過去研究發現，深層海水可以促進海藻生長，也可提升藻內活性物質含量，例如多醣類、抗氧化物質等等。

多醣體屬於醣類一種，是由一群複合的單醣聚合物組合成(分子量6,000-100,000），統稱（多醣），多醣體無法以化學方法來合成，主要是來自於菌體分泌或植物體。其功能具有抗病毒、抗腫瘤、抗氧化、抗發炎等活性，被視為最具潛力保健商品之一。過去研究發現，在藻類中含有許多陸生植物所缺乏的獨特性多醣成份，應用廣泛。本研究嘗試以深層海水培育長莖葡萄蕨藻(*Caulerpa Ientillifera*，通稱海葡萄)，萃取其海藻多醣並進一步探討對免疫細胞的活性影響。

2. 實驗方法：

取得以深層海水所培育約三個月的海葡萄，冷凍乾燥並研磨成粉末，以二次過濾水回溶，利用不同溫度 75℃、100℃、121℃及不同水量 20x、30x(藻粉/水；g/v)進行萃取，萃取時間 20 分鐘，找出最適萃取條件。萃取後的粗多醣以酒精沉降後以烘箱乾燥(40℃)，乾燥後於 4℃冷藏，以所得固體計算多醣萃取率(萃取率%=(乾燥粗多醣/每克乾燥海藻)*100)。本研究利用巨噬細胞進行免疫活性之檢測，先以 MTT assay 試驗海葡萄多醣對免疫細胞的生長影響，利用活細胞對 MTT 化合物的還原力來計算活存細胞率。免疫活性檢測中我們以脂多醣體(lipopolysaccharide，LPS)先行誘導巨噬細胞產生發炎反應，再添加不同濃度海葡萄粗多醣(0、50、100、150、200 g/ml)，在共軛顯微鏡下觀察巨噬細胞外觀，再檢測細胞內毒素一氧化氮(NO)生成量以評估海葡萄粗多醣對免疫細胞活性影響。

3. 結果與討論：

實驗結果，海葡萄最適萃取條件為 100℃、30x、20min，每克藻粉可萃取 20%的多醣含量。在 MTT 的試驗中，海葡萄多醣添加濃度在 50~150 g/ml 時有促進巨噬細胞生長現象，但是濃度 200 g/ml 時則細胞開始減少。進一步利用 LPS 誘導巨噬細胞產生發炎反應，巨噬細胞受到刺激時會產生偽足，以共軛顯微鏡觀察海葡萄多醣對巨噬細胞發炎反應的影響時顯示，加入多醣濃度(50~150 g/ml)時，巨噬細胞的偽足反應有減少現象，NO 也有相當低的生成量，但是在高濃度 200 g/ml 環境下，偽足現象增加，NO 生成量也增加了。

由上述實驗結果推論，巨噬細胞受到刺激發炎時，添加海葡萄多醣可抑制發炎反應，其多醣濃度須控制在(50~150 g/ml)，若多醣濃度高於此濃度範圍時，則不僅抑制巨噬細胞生長也促進發炎反應，此結果對未來海葡萄多醣的應用具有相當重要的參考價值。

P6. Effects of deep seawater on the immunocyte activity of *Caulerpa lentillifera* extract

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I. Introduction

Deep seawater (from a depth below 200 m) has a low temperature, abundant nutrients, high cleanliness, and is both pathogen-free and stable; thus, it can be applied to the foods, agriculture, and aquaculture industries. Previous studies have demonstrated that deep seawater not only promotes algal growth rates but also enhances algal polysaccharide content. Polysaccharides are high molecular weight (MW = 6,000~100,000) polymers of monosaccharides. Polysaccharides containing all the same monosaccharides are homopolysaccharides, whereas those that contain multiple types of monomers are heteropolysaccharides. In recent years, researchers have focused on isolating novel bioactive compounds from marine sources, examining potential benefits to human health. Marine algae are valuable sources of structurally diverse bioactive compounds. Polysaccharides are widespread in marine algae, exhibiting potential antioxidant, antiviral, anticancer and immunomodulating activities *in vitro* and *in vivo*. In this study, we examined how polysaccharides affected the activity levels of immunocytes, which were extracted from *Caulerpa lentillifera* by using a deep seawater culture.

II. Materials and Methods

C. lentillifera was cultivated in deep seawater for 3 months, washed using tap water, and freeze dried. To prepare defatted algal powder, the freeze dried algae were subsequently powdered using an abrader, dissolved in ddH2O, and extracted at various temperatures (75°C, 100°C, 121°C) and volumes of water (x20, x30 (algae powder/water; g/v)) for 20 min. The polysaccharide was precipitated using ethanol and dried in an oven at 40 °C.

The polysaccharide yield was calculated using the following formula: extraction rate % = (dried polysaccharide/g algae powder)*100. The macrophage survival rate was tested using an 3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, yellow tetrazole (MTT) assay. The macrophages were initially treated using lipopolysaccharide (LPS) and co-treated using polysaccharide; the immune activity levels were tested by assessing nitric oxide (NO) production and the appearance of cell pseudopodia.

III. Results and Discussion

The highest yield of *C. lentillifera* polysaccharides was extracted at 100°C, x30 (algal powder (g)/water (v)) at 20 min. The extraction rate was 20%. The MTT assay showed that polysaccharides treatments of approximately 50–150 mg/mL enhanced macrophage growth depending on the concentration and decreased macrophage growth when greater than 200 mg/mL was used. When the macrophages were exposed to LPS, NO release and pseudopodia were observed. The results also showed that polysaccharides decreased pseudopodia expression and NO release in macrophages at approximately 50–150 g/mL; however, polysaccharides increased pseudopodia expression and NO release in macrophages at levels greater than 200 g/mL.

The results show that *C. lentillifera* polysaccharides (50–150 mg/mL) yield anti-inflammatory effects in macrophages. At high concentrations (> 200 mg/mL), polysaccharides inhibit the growth of macrophages and promote inflammation. These results are critical for future applications of *C. lentillifera* polysaccharides.

P7. 低溫溫室培育山葵之胺基酸組成與抗氧化性研究

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蔡忠良(驚奇的哇沙咪實業有限公司)

1. 前言

山葵(*Wasabia japonica*)，十字花科植物，經濟價值高，在日本也被稱為綠色黃金。台灣自日本引進種植山葵已有數十年歷史，由於山葵是高經濟作物，因此在阿里山大量種植但也帶來許多環境的問題，國內有民間企業研發新式栽種技術，將山葵移植到平地種植，利用低溫溫室並選育耐室溫環境之山葵大量種植，不僅創造高經濟價值外，更可避免對山林造成衝擊。但低溫溫室需耗損能源以維持低溫環境，本研究將模擬深層海水低溫冷能應用技術建置低溫溫室種植山葵並分析其胺基酸組成與抗氧化力以作為後續開發深層海水冷能應用之參考。

2. 材料與方法

利用低溫冰水機模擬深層海水冷能利用模組建置低溫溫室(18~25℃)種植山葵。並將培育一年以上之山葵取其莖部進行胺基酸成分分析，以離子交換層析法進行定量分析。再者，分別利用 100℃熱水(HWE)、40℃水萃(HE)、95%酒精萃取(EE)，得其萃取物以分析抗氧化力。抗氧化分析則包含 DPPH 自由基清除力與亞鐵離子螯合力等。

3. 結果與討論

研究結果顯示，低溫溫室內種植之山葵含有 27 種游離胺基酸成分，其中含有 8 種人體所需之必需胺基酸。抗氧化力分析結果顯示，DPPH 自由基清除力以 95%酒精萃取物具有最佳抗氧化力(EC50 為 11.9mg/ml)；亞鐵離子螯合力則以水萃物含有較佳之抗氧化力。此結果顯示水萃物與酒精萃取物分別具有不同抗氧化作用之能力。

山葵營養豐富，未來更可開發多元化加值產品如山葵酒、山葵醋、山葵保養品等，擴大山葵全株利用性，提升山葵產業價值。

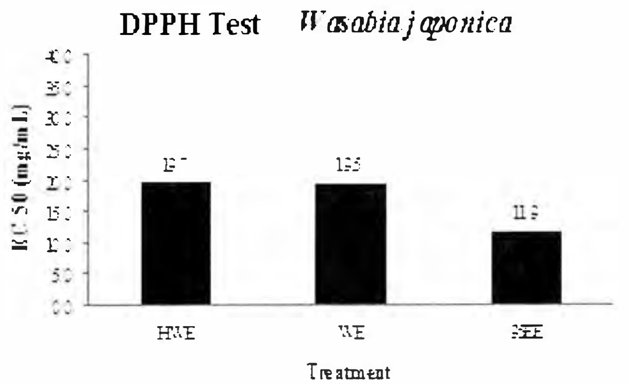

Fig 1. DPPH free radical scavenging activity assay of *Wasabia japonica*

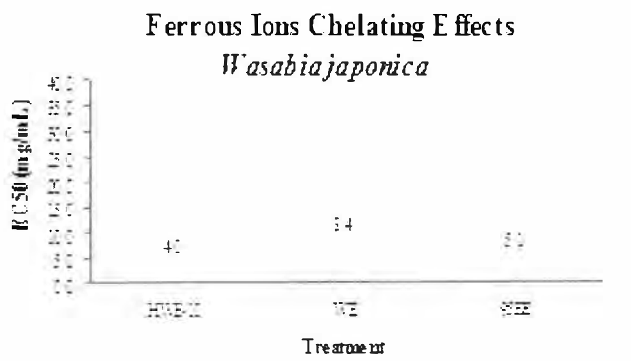

Fig 2. Ferrous Ions Chelating Effects of *Wasabia japonica*

P7. Amino acids content and antioxidant capacity of "*wasabi*" grown under below-room-temperature greenhouses

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I. Introduction

"*Wasabi*" (*Wasabia japonica*) is one of cruciferous plants and of high economic value. It is also known as a green gold in Japan. It was introduced into Taiwan several decades ago. It has been grown in the Ali Mountain and it has adversely affected the environment thereof. New cultivation technology (including the use of below-room-temperature greenhouses and the selection of the "*wasabi*" that can ensure room temperatures) has allowed "*wasabi*" to grow on sea-level plains to raise profit levels and to eliminate the adverse impacts on the forest environment. However, energy is needed to maintain chill greenhouses to below the room temperature. In this study, the amino acids content and antioxidant capacity of "*wasabi*" grown under below-room-temperature greenhouses chilled by the use of the deep seawater were tested. This study will be used as a reference for the future development of the deep seawater application technology.

II. Materials and methods

"*Wasab*i" is grown under below-room-temperature (18 to 25 °C) greenhouses which are chilled by the use of the deep seawater. First, quantitative analysis through ion exchange chromatography was used on stem portions of "*wasabi*" that has been grown for more than one year to determine its amino acids content. Also, hot water at 100 °C, 40 °C water extraction method and 95% alcohol extraction method are used to obtain extracts so as to analyze its antioxidant capacity, which includes DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical removal capacity and ferrous ion chelating ability.

III. Results and Discussion

According to our lab tests, "*wasabi*" grown under a below-room-temperature greenhouse contains 27 types of free amino acids, 8 of which are needed by human body. In addition, extract obtained by the 95% alcohol extraction has the highest level of antioxidant capacity (EC_{50} was 11.9 mg/ml). Regarding ferrous ion chelating ability, extract obtained by the water extraction has a high level of antioxidant capacity. Therefore, extract obtained by the 95% alcohol extraction and the extract obtained by the water extraction have different levels of antioxidant capacity.

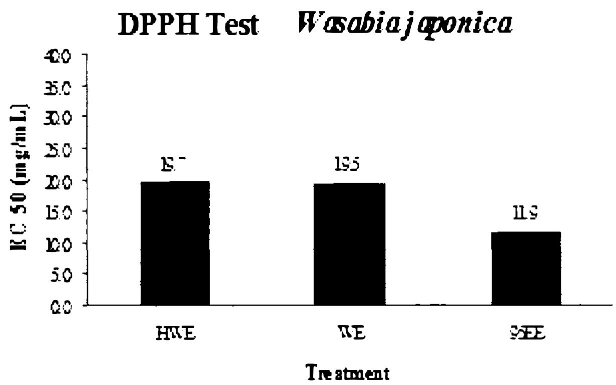

Fig 1. DPPH free radical scavenging activity assay of *Wasabia japonica*

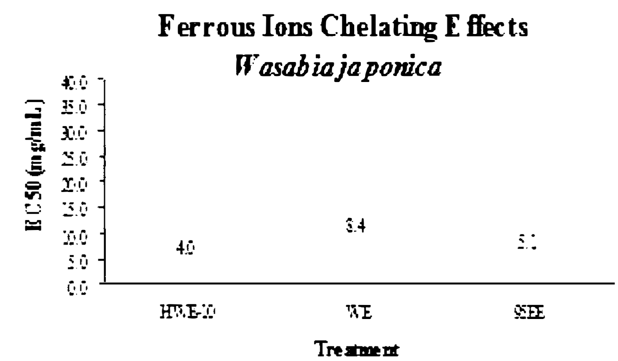

Fig 2. Ferrous ions chelating effects of *Wasabia japonica*

P8. 深層海水對茼蒿水耕栽種之應用

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1. 前言

近年來深層海水 (DSW) 不止應用於醫藥產業上也應用於農業上。有文獻報導指出，將螺旋藻培育於深層海水培養液中，其萃取物可以有效抑制各種不同的人類癌細胞生長，其癌細胞毒殺效果與添加濃度呈正相關。另一應用深層海水的國家日本，則是於農業應用上以深層海水生產高品質的蔬果與作物。

蔬菜茼蒿原產地位於地中海和東亞，為一種食用葉菜類，適合生長於微寒的氣候區。在台灣，茼蒿盛產於冬天，而且台灣民眾喜愛於冬天吃火鍋時添加茼蒿一起食用。在本研究中，嘗試以深層海水水耕方式來栽種茼蒿，並觀察深層海水添加與否對茼蒿根、莖和葉的生長是否有影響。

2. 材料與方法

本實驗中所使用之去鹽深層海水濃縮液 (DS-DSW) 購自於台灣海洋深層水公司。實驗中將發芽之茼蒿幼苗以含/不含深層海水濃縮液之水耕液 (HCS) 栽種，於溫度設定 25 ± 0.5℃和日照 12 小時/黑暗 12 小時週期循環之植物培養箱中培育 60 天。此實驗用茼蒿共分成五組，其中一組為對照組，僅使用不含深層海水之水耕液栽種茼蒿；另四組實驗組分別為 10^{-4} DSW 組、10^{-5} DSW 組、10^{-6} DSW 組和 10^{-7} DSW 組，其栽種茼蒿的水耕液中分別含有萬倍、十萬倍、百萬倍和千萬倍體積稀釋之去鹽深層海水濃縮液。

3. 結果

從表一的結果中可顯示深層海水應用於茼蒿生長上的效果，雖然過高濃度的深層海水添加會抑制茼蒿的根部和葉部生長，但是如能添加適當濃度的深層海水，將其當一水耕肥料使用，則不會對茼蒿的根部和葉部生長產生抑制作用，反而對茼蒿的葉部生長有助益。以 10^{-7} DSW 組 (平均葉片數 13.6 片)和對照組(平均葉片數 13.6 片)相比較，此結果顯示適當濃度的深層海水添加明顯的增加了 17% 的葉片數。

表一、茼蒿以含/不含深層海水之水耕液培養 60 天後生長情形

組別	根長 (公分)	莖長 (公分)	葉片數 (片)
控制組	13.5 ± 6.3	1.7 ± 0.5	11.6 ± 1.7
10^{-4} DSW 組	4.1 ± 3.8	2.2 ± 0.5	6.8 ± 1.0
10^{-5} DSW 組	9.6 ± 2.3	1.8 ± 0.6	9.7 ± 1.7
10^{-6} DSW 組	8.8 ± 3.8	1.8 ± 0.7	11.3 ± 2.6
10^{-7} DSW 組	12.1 ± 5.4	1.8 ± 0.4	13.6 ± 3.0

P8. The Application of Deep Sea Water for Hydroponically Cultivated *Chrysanthemum coronarium*

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I. Introduction

In recent years, deep sea water (DSW) has been applied not only in medicine but also in agriculture. Literature research showed that the extract of *Spirulina maxima* cultured in DSW effectively inhibited various human cancer cells with concentration dependency. In Japan, DSW has been applied to product high quality vegetable and crop for agriculture.

The garland chrysanthemum (*Chrysanthemum coronarium*) is native to the Mediterranean and East Asia. The garland is used as a leaf vegetable, and it grows well in mild or slightly cold climates. In Taiwan, the garland is abundant in winter and is often added in hot pot dishes. In this study, hydroponically cultivated garland with DSW was investigated on the basis of the growth rates of the root, stem and leaf.

II. Materials and Methods

The desalted concentrated DSW (DS-DSW) was purchased from Taiwan Deep Ocean Water Co., Ltd. The germinated garlands were supplied in hydroponically cultivated solution (HCS) with/without DSW, and they were placed in an incubator maintained at 25 ± 0.5℃ and 12/12 hr photoperiod for 60 d. The garlands were divided into 5 groups with different cultured solutions, with control group cultured only using HCS, while 10^{-4} DSW, 10^{-5} DSW, 10^{-6} DSW and 10^{-7} DSW groups were cultured with DS-DSW diluted $1:10^4$, $1:10^5$, $1:10^6$ and $1:10^7$ in HCS, respectively.

III. Results

Results in Table 1 showed the effect of DSW application on vegetative growth of the garlands. The data indicated that DSW of high concentration resulted to significant inhibitions on vegetative growths of root and leaf, but DSW of suitable concentration did not. The 10^{-7} DSW group had significant effects on vegetative growths of leaf number than that of the control group (about 17%).

Table 1. Growth of garland in HCS with/without DSW after 60 d

Group	Root (cm)	Stem (cm)	Leaf number
control	13.5 ± 6.3	1.7 ± 0.5	11.6 ± 1.7
10^{-4} DSW	4.1 ± 3.8	2.2 ± 0.5	6.8 ±1.0
10^{-5} DSW	9.6 ± 2.3	1.8 ± 0.6	9.7 ± 1.7
10^{-6} DSW	8.8 ± 3.8	1.8 ± 0.7	11.3 ± 2.6
10^{-7} DSW	12.1 ± 5.4	1.8 ± 0.4	13.6 ± 3.0

P9. 深層海水對苜蓿芽生長之正向影響

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1. 前言

深層海水(DSW)的特色是乾淨、低溫、含豐富的礦物質和穩定的水質，因此，深層海水很適合用來當作培育植物生長的添加物質，特別是其含有各式各樣的營養物質。苜蓿是常見的一種生菜，常被用來製作三明治、捲餅和生菜沙拉，其含有豐富的營養成分，如維生素 A、B、C 和 K，鈣、鎂、鉀、鐵和磷等成分。常食用苜蓿可減少疲勞感，降低膽固醇和維持血糖平穩。此外，有文獻報導深層海水不止可加速疲勞身體的恢復，還可以增加植物的生長，甚至是加強藥用植物的生物活性。在此次的實驗中，苜蓿將以不同濃度的深層海水培養後，每天觀測紀錄其生長高度和重量的變化。

2. 材料與方法

於台灣海洋深層水公司購買培育苜蓿實驗所使用的去鹽深層海水濃縮液 (DSW)。首先，實驗苜蓿被依使用不同濃度的深層海水培養液而分成五組，對照組的苜蓿是僅使用逆滲透水(RO)培育，實驗組的苜蓿則依其深層海水培養液濃度不同分成10^{-3} DSW、10^{-4} DSW,、10^{-5} DSW 和 10^{-6} DSW四組，分別將去鹽深層海水濃縮液於逆滲透水中稀釋千倍、萬倍、十萬倍和百萬倍。實驗苜蓿於25 ± 0.5 ℃的植物培養箱中進行黑暗培養。

3. 結果

表一的結果顯示深層海水對於苜蓿生長有明顯的效果。在培養的第六天，10^{-3} DSW 和 10^{-4} DSW 兩組苜蓿平均生長長度皆為 6.4 ± 0.1 公分，與對照組相比 (5.9 ± 0.1 公分)，兩組長度皆多了 8%。雖然 10^{-3} DSW 組長度較對照組長，但在重量上兩組的結果幾乎同重，表示 10^{-3} DSW 組苜蓿生長較為細長，因此其重量上變化不大。而在培養第六天的 10^{-4} DSW 組，不僅是長度較對照組長，而且重量也較對照組多 17%，表示 10^{-4} DSW 組的苜蓿生長又高又壯，因此在重量上增加許多。這些實驗結果都顯示出適當濃度的深層海水對於植物的生長和品質有著正面的加分效果。

表一、於深層海水溶液中栽種苜蓿之長度

組別	第二天(公分)	第四天(公分)	第六天(公分)
control	1.6 ± 0.06	3.9 ± 0.08	5.9 ± 0.10
10^{-3} DSW	1.9 ± 0.15	4.7 ± 0.05	6.4 ± 0.10
10^{-4} DSW	2.0 ± 0.13	4.6 ± 0.12	6.4 ± 0.10
10^{-5} DSW	1.9 ± 0.10	3.9 ± 0.10	5.9 ± 0.05
10^{-6} DSW	1.6 ± 0.05	3.0 ± 0.06	5.9 ± 0.10

P9. Positive Effects of Deep Sea Water on the Growth of Alfalfa (*Medicago sativa*)

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I. Introduction

Deep sea water (DSW) is characterized by its clarity, low temperature, abundant nutrients and stable water quality. Thus, DSW would be a good substrate for vegetable growth since it contains various nutrients. Alfalfa is often used in sandwiches, burritos and salads. Alfalfa contains a wide array of vitamins and minerals, including vitamin A, vitamin B, vitamin C, vitamin K, calcium, magnesium, potassium, iron and phosphorus. Eating alfalfa can reduce fatigue and cholesterol, and maintain blood sugar levels. Furthermore, some reports showed that DSW not only accelerates recovery from physical fatigue, but also increases the growth of plant as well as enhances the bioactivity of medicinal plants. In this study, alfalfa was cultured in different concentrations of DSW, and their length and weight were recorded every day.

II. Materials and Methods

The desalted concentrated (DS) DSW was purchased from Taiwan Yes Deep Ocean Water Co., Ltd. Experimental alfalfa was divided into 5 groups treated with different concentrations of DSW. The control group was grown only with reversed osmosis water (RO), and DSW groups were grown using RO with DSW. Groups of 10^{-3} DSW, 10^{-4} DSW, 10^{-5} DSW and 10^{-6} DSW indicated that alfalfa was cultured with DS-DSW diluted $1{:}10^3$, $1{:}10^4$, $1{:}10^5$ and $1{:}10^6$ in RO, respectively. All groups were placed in an incubator maintained at 25 ± 0.5℃ in the dark.

III. Results

Table 1 shows that DSW had a significant effect on alfalfa growths. On the sixth day, the average lengths of 10^{-3} and 10^{-4} DSW groups were 6.4 ± 0.1 cm, exhibiting 8% better length than that of control group (5.9 ± 0.1 cm). Although alfalfa of 10^{-3} DSW group had better length than the control, both weights were almost the same. Furthermore, 10^{-4} DSW group showed that the weight was 17% more than control group on the sixth day. The result indicated a positive effect of suitable concentration of DSW on growth and quality of alfalfa.

Table 1. Length of alfalfa cultured in different DSW solutions

group	2th day (cm)	4th day (cm)	6th day (cm)
RO	1.6 ± 0.06	3.9 ± 0.08	5.9 ± 0.10
10^{-3} DSW	1.9 ± 0.15	4.7 ± 0.05	6.4 ± 0.10
10^{-4} DSW	2.0 ± 0.13	4.6 ± 0.12	6.4 ± 0.10
10^{-5} DSW	1.9 ± 0.10	3.9 ± 0.10	5.9 ± 0.05
10^{-6} DSW	1.6 ± 0.05	3.0 ± 0.06	5.9 ± 0.10

P10. 深層海水冷能運用於竹笙培育技術開發

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1. 前言

竹笙(*D. echinovolvata*)是一種營養成分豐富的保健食品，含有豐富的蛋白質~20%、碳水化合物、多種氨基酸(含 8 種人體所需的胺基酸)以及維生素，是一種健康無污染的綠色食品，分佈區包括了日本、中國、北美等，台灣竹林地也有少量野生種竹笙，食用則全仰賴大陸進口。

竹笙屬於腐生菌需要高腐植質的生長環境，且於菌絲體培育環境需要一恆溫環境(~25 °C)，而於子實體培育階段，需要日夜溫差(6-8 °C)刺激子實體出現，因此台灣野生多出現於山區，而以大陸福州為例，且子實體培育每年採收一期(4-6 月)，以符合竹笙子實體生長環境所需。因此本研究運用東部深層海水其冷能特性，將熱交換後冷的淡水應用於控制室內培育種植環境所需的溫度與溼度，避免冷能的浪費，達到省能源式的室內培育高價值菇菌類產品的目標。

2. 實驗方法

以冰水機模擬深層海水低溫特性，建置控溫環境系統。

(1)竹笙菌絲體培育階段：溫度控制於 25 °C，濕度控制~60%。

(2)竹笙菌絲體培育階段：白天 26-29 °C，晚上 20-22℃（日夜溫差 6-8℃)。

3. 結果與討論

運用種植菇菌類的廢料及稻草農業廢棄物之的環保型營養源，建立竹笙培育條件與探討，完整竹笙培育步驟包括母菌種培養與菌絲體培養(約 60 天完成)、覆土進行子實體培育、竹笙蛋生成(覆土後約一月出現)、竹笙子實體出菇(竹笙蛋成熟至出菇約 3 周)，詳如圖一所示。

而於菌絲體培養部分，在相同營養源條件下，菌絲成長速率與室內溫度控制穩定性呈現相關性，於 25±1°C 的植物生長箱內，菌絲生長速率為 3.5-6 cm/周，於 24±3°C 的 10 坪竹笙培養室內，生長速率 2.5cm/周，呈現控溫誤差越小，較益菌絲體成長。

而於子實體培育部分，南部地區 4-5 月間日夜溫差為 6-8°C，符合竹笙子實體成長條件，反觀目前室內竹笙培育室採用內溫室且 PP 不透光材質，保溫效果好日夜差異只約 2-3℃。因此正透過程式化控溫與液-氣熱交換模組的改善，加強培養室夜間溫低控制，預期達到日夜溫差為 6-8°C 的目標，希望透過仿自然出菇條件複製至室內環控，預期達到一年可多次(>3 次）採收的目標，有別於人工戶外子實體培育，一年只採收一次之高成本缺點。

圖一、竹笙生長外觀圖。(A)母菌種培養、(B)太空包菌絲體培養、(C)覆土進行子實體培育、(D)竹笙蛋外觀圖 (E)竹笙子實體外觀圖。

P10. Application of cold energy from deep sea water for the cultivation of bamboo fungus

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I. introduction

The bamboo fungus (*Dictyophora echinovolvata*) is a nutrient-rich food, including protein ~20%, carbohydrates, amino acids (8 kinds of essential amino acids) and vitamins. They are widely distributed in Japan, China and North America. A small amount of wild bamboo fungus has been observed in Taiwan's bamboo areas. Daily supply is totally dependent on imports from China.

The growth of the mycelium of the bamboo fungus needs a constant temperature environment (~25°C), while in the fruiting body cultivation stage, day and night temperature difference (ΔT=6-8°C) was needed to stimulate the growth of the fruiting bodies. Therefore, the wild bamboo fungus mostly grows in the mountainous areas of Taiwan. In this study the cold energy of deep sea water (DSW) passing through the cold-heat exchanger was applied to control the temperature and humidity of the indoor cultivation environment. This energy-saving indoor cultivation process was used for high-value mushrooms to avoid the waste of cold energy.

II. Materials and Methods

Water chiller and temperature control systems were used to simulate low temperature properties of the DSW. (1) For mycelium cultivation stage: temperature was set at 25 °C, humidity was controlled to 60%. (2) for fruiting body cultivation stage: day-time temperature was set at 26-29°C, night-time temperature was set at 20-22°C.

III. Results and discussion

The detailed steps for bamboo fungus cultivation are shown in Fig. 1. During mycelium culture, mycelial growth rate was related to the stability of indoor temperature. Mycelial growth rate was 3.5-6 cm/week at 25±1°C. However, at 24±3°C, growth rate was 2.5cm /week.

April-May period was suitable for fruiting body outdoor cultivation in Taiwan southern region. However, the temperature difference between day and night is about 2-3°C in our indoor cultivation room due to insulation effect. Therefore, programmable temperature control with liquid-gas heat exchange module was used to improve the situation. This simulation of natural conditions was expected to produce a harvest 3 times a year.

Fig. 1、Bamboo fungus cultivation。(A) fungus cultivation (2W)、(B) mycelium cultivation (4-6W)、(C) fruiting body cultivation process for 4W and eggs-like stage of bamboo fungus (D), (E) after around 3W, fruiting bodies could be observed.

P11. 利用三原色（RGB）分析法測量於平板光生物反應器中以深層海水培養之藍綠藻 *Spirulina platensis* 的藻藍素含量

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1、前言

由於暖化與氣候變遷，減碳已成為世界各國刻不容緩的課題。近年來，以螺旋藻進行微藻固碳日漸受到重視，基於藍綠菌具有高藻體生長速率的特性，可有效以光合作用固定二氧化碳並轉化成藻體 biomass，此外，螺旋藻富含藻藍素，藻藍素(C-PC)為藍綠菌體內三種藻膽蛋白的一種，藻藍素為捕光色素蛋白，有效捕捉葉綠素較難吸收之波長，可作抗氧化藥物、染劑以及抗菌劑等應用，因此以螺旋藻進行生物固碳並同時生產藻藍素，具有相當高的發展潛力。

2、材料與方法

本研究利用 TL5 日光燈管進行全日光照，並以 0.2 vvm 之曝氣速率通入 2.5%之 CO_2 氣體。每日進行藻液取樣，以 pH meter 測量其酸鹼值，並以分光光度儀(UV-VIS)於吸收波長 680 nm 下測定其吸光值，再將之換算成藻體細胞乾重以得藻體濃度。藻體生長後將之離心並凍乾成乾燥藻粉。

3、結果與討論

本研究主要建構一套快速測量藻體中藻藍素含量之三原色(RGB)分析方法，光自營藍綠藻(*Spirulina platensis*)被選用當做測試藻種，並利用平板光生物反應器以及深層海水進行培養。三原色分析方法主要是基於微藻在生長的過程中，其組成變化會伴隨著顏色之變化，這些顏色變化可以從三個主要的顏色來測量，分別是紅、綠、藍三個顏色，本研究乃利用這個特性建構出一個線性函數來表示藍綠藻中藻藍素的含量，以建立簡便快速的螺旋藻藻藍素含量測定方法。研究結果顯示，利用三原色分析法回歸得到的校正曲線 R^2 在 0.995 以上，且從三原色分析法預估的藻藍素含量與實際測量的藻藍素含量大約僅有 2.1%至 5.2% 之些微偏差，顯示利用三原色分析法來測量藍綠藻 *Spirulina platensis* 內的藻藍素含量應是可行的。

關鍵字：微藻、*Spirulina platensis*、藻藍素、三原色分析、平板光生物反應器

P11. Rapid estimation of C-phycocyanin production of *Spirulina platensis* by RGB analysis in a plate-type photobioreactor

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Introduction

Biofixation of CO_2 achieved by microalgae and cyanobacteria has gradually raised attention around the world owing to its efficient CO_2 removal rate; beside, the microalgae biomass could be applied to various downstream applications like biofuel, carbohydrate, pigments, proteins production, etc. *Spirulina* is considered a good candidate for CO_2 biofixation in a culturing system; besides, *Spirulina* is rich in C-phycocyanin (C-PC), which could be utilized in colorants, diagnosis reagent, nutritious supplements, and pharmaceuticals. Therefore, using *Spirulina platensis* to assimilate CO_2 as the carbon source with simultaneous production of valuable C-phycocyanin products gives great benefits.

Materials and Methods

The microalgal strain (i.e., *Spirulina platensis*) used in this work was isolated from a freshwater area located in southern Taiwan. The modified Zarrouk medium was used to cultivate the alga. *S. platensis* culture was grown at 32°C under a one side light intensity of 700 μmol/m^2/s (illuminated by TL5) in the flat type photobioreactor

Results and Discussion

In this study, a colorimetric analysis method was developed to rapidly determine the C-phycocyanin content of microalgae. *Spirulina platensis*, which is a common producer of C-phycocyanin, was chosen as the model organism, since it is well known to accumulate high levels of intracellular protein C-phycocyanin in deep seawater supplemented medium in a plate-type photobioreactor. The color variation of microalgae during the period of cultivation evaluated by the response of the three primary colors (red, green, and blue) was modeled with a linear correlation function (RGB model), which can be used to accurately estimate the microalgal C-phycocyanin content with a R^2 value of 0.995. It is also crucial to justify whether the RGB analysis can be used in determining the C-phycocyanin content of real microalgae samples during the cultivation. It was found that the deviation between the predicted values and experimental results was around 2.1-5.2%, which is acceptable from practical aspects. Therefore, this work seems to demonstrate the feasibility of using RGB analysis for rapid determination of C-phycocyanin content of *Spirulina platensis*.

KEYWORDS: *Spirulina platensis*; C-phycocyanin; RGB analysis; plate-type photobioreactor

P12. Effects of aquatic body walking using deep ocean water on stress and blood pressure

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Ⅰ. Introduction

This study aims to give a therapeutic model to manage stress and present concrete data to support the feasibility of the model. To this end we analysed and compared the stress and blood pressure changes resulting from water walking using deep sea water.

Ⅱ. Experimental Design

As subjects of this study, 20 female college students in Sokcho city, Korea took part in the exercise for two weeks. The duration of the exercise was from the 3rd of December, 2011 to the 14th of December excluding weekends, with 10 total exercises performed. We applied Salivette® (Sarstedt, Germany) which is handy and lessens inducing stress and analysed with Enzyme-linked immunoassay (EIA). SANKEI meta blood pressure meter (SANKEI Co, Japan) was used to measure the diastolic and the systolic blood pressure.

Ⅲ. Results & Discussion

In this study we quantified and analysed saliva cortisol and blood pressure to find any possible interrelationships between aquatic body walking and stress reduction as well as blood pressure of the human body. What counts in stress is not the source of tension but how to handle the stress level in an appropriate manner. In that vein, Watsu, a soothing type of massage that takes place in warm, waist-deep water, with the purpose of soothing stress and relieving nervousness led to the reduction in saliva cortisol. Systolic blood pressure was shown to decrease with the reduction of cortisol, which is identical to the results of previous researches. An experiment involving secondary school students reported that the reduction in stress level affected the noticeable decrease in systolic blood pressure. So the relief and relaxation in the body and the mind due to the reduction of stress level are certain to affectthe decrease in systolic blood pressure. In this respect Watsu in deep ocean water can be an effective remedial solution to reduce tension and relieve nervousness.

Ⅳ. Conclusion

From this study we applied Watsu program to the subjects of our experiment to shed light on the correlations between aquatic body walking exercise and the reduction of stress hormone, the salivary cortisol and blood pressure change. The results of the experiment found positive effects of Watsu in stress reduction and blood pressure management. So Watsu is considered to be an effective way to reduce stress and the therapeutic effect of Watsu can ultimately contribute to a well balanced body. Additionally Watsu is easy to utilize and practice without any special equipment or tools so this economic approach will work for patients wanting relief in their mind and body with the help of the therapist as well as ordinary people who suffer from stressful environments.

P13. 以聚合酶鏈鎖反應技術鑑定海馬細菌性病原

°陳明慧、時雨青、張景煇(工研院)

I. 前言

海馬是為一種傳統中藥藥方，屬於高經濟價值之魚類。由於 2004 年華盛頓公約將所有海馬品種列入附錄 II 名冊，所有的進出口海馬都必須有產地證明才可以交易。因此，台灣積極發展海馬養殖技術，但海馬對於水質生長環境要求高，而深層海水的潔淨特性提供海養生長優質的環境 然而，海馬卻容易因疾病導致死亡影響養殖產量。因此，海馬病原之鑑定與檢測有其必要性。

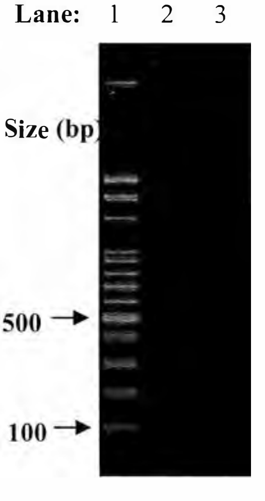
哈維氏弧菌(*Vibrio harveyi)*是海馬中發現的常見病原菌，以傳統方法鑑定病原菌非常耗時，因此，開發一種有效又快速的檢測方法是十分重要的。本研究將以聚合酶鏈鎖反應技術(Polymerase Chain Reaction, PCR)來鑑定海馬細菌性病原。

II. 材料與方法

本實驗所使用之標準菌株為 *V. harveyi* BCRC 12907。以商業套組進行 DNA 之萃取。於 0.2 ml 微量離心管加入 PCR 反應：Prozyme PCR buffer (1×buffer: 10 mM Tris-HCl, pH 8.8; 1.5 mM $MgCl_2$; 50 mM KCl and 0.1% Triton X-100) 各 200 µM 之 dATP、dGTP、dCTP 、dTTP ，1 µM 之引子組， 0.4 unit 之 Prozyme DNA polymerase, 再加入 1 µl 之模版 DNA，使總量為 20 µl。而後將微量離心管置入 PCR thermocycler, 進行 PCR 反應，vhtoxR-F1/ vhtoxR-R1 引子組之 PCR 反應條件為 94℃加熱 5 分鐘後，以 94 ℃、30 秒；40℃、30 秒；72℃、1 分進行 30 cycles, 最後以 72℃維持 10 分鐘;。反應完後, 取 5 µl 之 PCR 產物，以 2 % agarose 於 0.5X TAE buffer 中進行電泳分析，經 ethidium bromide 染色，以影像處理系統觀察後拍照。

III. 結果與討論

本研究利用 PCR 進行哈威氏弧菌之鑑定，以 vhtoxR-F / vhtoxR-R 進行 PCR 反應，PCR 反應後經電泳確認結果，結果顯示可有效檢測出 382 bp 之預期產物，如圖一所示，
因此，PCR 可作為快速檢測哈威氏弧菌的技術。



圖一. 以 PCR 進行哈威氏弧菌之鑑定之結果
Lane 1: 100 bp ladder
Lane 2: PCR product amplified from *V. harveyi* BCRC 12907 by using vhtoxR-F / vhtoxR-R primer set
Lane 3: blank control

P13. Identification of Bacterial Pathogen in Seahorse by PCR

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(Green Energy & Eco-Technology System Center, ITRI Southern Region Campus, Tainan, Taiwan)

I. Introduction

The seahorse is a highly economically valuable fish for Chinese traditional medicine. According to the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), all kinds of seahorses are listed on Appendix II, and the origin certificate of seahorses should be provided before importing and exporting of seahorses. Thus, the technology of seahorse aquaculture is proactively developing in Taiwan. In addition, the water quality of the aquaculture environment is important to seahorse growth. The cleanliness of deep sea water (DSW) provides an excellent condition for seahorse growth. However, the production of seahorse may be affected by aquatic diseases. Therefore, it is necessary to identify and detect the pathogens of seahorses.

Vibrio harveyi is the most common pathogen found in seahorses. Traditional methods for identification of marine pathogens are time-consuming, thus, it is important to develop an effective and rapid detection method. The PCR method was carried out in this study.

II. Materials and Methods

Bacterial strain *V. harveyi* BCRC 12907 was used in this study. Total genomic DNA of *V. harveyi* was prepared according to the instructions of PureLink™ Genomic DNA Mini Kit (Invitrogen, UK).

The PCR reaction mixtures contained 10 pmol of vhtoxR-F1/ vhtoxR-R1 primers, 200 μM each of deoxyribonucleoside triphosphate, 10 mM Tris–HCl (pH 8.8), 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100, 0.4 U of thermostable DNA polymerase, and 1 μl of the cell-free DNA, in a final volume of 20 μl. The PCR mixtures were then subjected to 94°C for 5 min followed by 30 cycles of 94°C for 30 sec, 40°C for 30 sec and 72°C for 1 min, and after amplification an extended step of 10 min at 72°C. The PCR products were confirmed by 2% agarose gel electrophoresis.

III. Results and Discussion

Figure 1 showed that the primer set generated PCR products with expected molecular size of 382 bp. By using the vhtoxR-F1/ vhtoxR-R1 primers, only DNA from *V. harveyi* generated the expected PCR products. Our results demonstrated that the PCR allowed the rapid detection of *V. harveyi*.

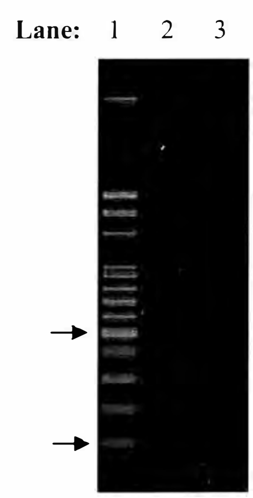

Figure 1. PCR results for *V. harveyi* identification
Lane 1: 100 bp ladder
Lane 2: PCR product amplified from *V. harveyi* BCRC 12907 by using vhtoxR-F / vhtoxR-R primer set
Lane 3: blank control without cells

P14. Application of desalination of seawater with saturated vapor pressure

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Reduced pressure evaporation is the principle of lowering the boiling point of water by reducing the pressure. It makes use of the relationship between temperature and pressure to reduce energy consumption needed for elevating temperature. As the result, the highest evaporation was observed at 80 ℃ and -40cmHg after applying vapor pressure depending on different seawater temperatures. It was found that the higher concentration in the factor test experiment depending on seawater concentration induced higher evaporation. Also, in the factor test experiment, the amount of evaporation increased as the pressure decreased. It is concluded that the most important factor of evaporation was concentration, followed by temperature, and the least important factor was pressure. Through this experiment, optimized conditions of desalination with reduced pressure evaporation device were verified.

P15. 包裝飲用水導電度分析與深層海水濃縮液應用於逆滲透飲用水水質改善研究

°孫志誠(永達技術學院)

1. 前言

飲用水處理技術之發展，源於人類為獲得優質及安全的飲水，所發展出的科技程序。畢竟，飲用水關係著人們身體的健康，人類身體 70%以上為水，因而飲水對人類而言是相當重要的。因此，古人有云『水為百藥之王』。

飲用水處理技術由早期的傳統物理過濾程序，演進至今日之高分子薄膜的精密過濾程序[1,2]。早期因原水污染程度有限，傳統的物理過濾即可因應飲用水水質所需，但隨著為工業發展，都會區人口的密集，工業污染及農畜污染，使得原水水質快速惡化。自來水公司提供之自來水雖符合飲用水水質標準，但仍有諸多問題是無法解決的，市井小民仍尋求各種高科技的程序，確保自己飲用水的安全問題。其中包裝水的購買以及逆滲透(RO)淨水設備為近年來發展最為普遍之飲用水供應方式。

包裝水為應用各種淨水設備將不同水源之水經處理後，包裝成瓶裝、桶裝以及杯裝水進行販售，優點為方便易購得，且有銷售公司的安全保障，缺點為飲用包裝水的人們經濟的負擔較重，以及產品品管及公司信用等問題。一般民眾大多選擇相信廠商，或是以價廉為考量，一般文獻亦鮮少進行市售包裝水之評估，故而有需要進行研究探討。

逆滲透(RO)淨水設備之所以普及首歸於研發技術之提升，次歸功於價格的價低、水質污染之嚴重、水質硬度之問題等。迫使民眾需求純淨之飲用水，其優點為可獲得純淨之飲用水外，但另一方面消費大眾亦有水質太過純淨之疑慮出現，改質 RO 純水使其兼顧純淨與含礦物質亦是值得探討之問題。

市售之包裝水無非是以礦泉水、純水、海洋生成水、海洋深層水、蒸餾水等名稱自居，但皆無標示最易顯示出礦物質含量之導電度值，故以實驗室之導電度計對市售之包裝水進行量測及分析研究。另外，RO 純水導電度之提升亦是本研究之探對課題。

2. 實驗方法

2-1 導電度及 pH 值量測

於不同水樣中各取出 5ml，置入 30ml 樣品瓶中，將導電度計之電極置入樣品瓶中，進行導電度之量測。另再將 pH 電極置入樣品瓶中，由 pH 計讀取測試水樣之 pH 值。

2-2 活性碳或麥飯石添加於 RO 純水後導電度值量測

將精秤之活性碳或麥飯石置入裝有體積 100ml RO 純水之燒杯中，再將燒杯置於電磁攪拌機上，以攪拌子均勻攪拌燒杯中之 RO 純水與活性碳或麥飯石，固定時間間隔量測燒杯中水樣之導電度值。

2-3 RO 純水添加深層海水濃縮液之導電度量測

將 500 ml RO 純水置入燒杯中，逐次加入深層海水濃縮液，記錄加入之體積，進行導電度量測。

3. 實驗材料

市售礦泉水包含:大西洋蒸餾水(大西洋飲料),百年好水(台鹽),健康純水(喝好水),樂活主張(可口可樂)悅氏(名牌食品)台鹽海洋水(台鹽)波爾(金車)多喝水(味丹)竹碳水(味丹)深命力（台灣海洋深層水）。蒸餾水及 RO 純水則由實驗室製得。

4. 結果與討論

4-1 市售包裝水導電度量測值與 RO 純水及蒸餾水之比較

	導電度	PH
(1)蒸餾	1.43	6.56
(2)RO	6.33	6.23
(3)大西洋	1.86	7.82
(4)百年好水	4.07	6.27
(5)健康純水	10.27	8.71
(6)樂活主張	25.7	7.53
(7)悅氏	38	7.49
(8)台鹽海洋	42.2	8.48
(9)波爾	153.8	7.43
(10)多喝水	247	7.1
(11)竹炭水	266	7.46
(12)深命力	336	6.12

4-2 活性碳及麥飯石添加對 RO 純水導電度之影響

RO 純水於活性碳中之滯留，可有效的提升 RO 純水之導電度值。RO 純水於麥飯石中之滯留，對於 RO 純水之導電度值提升相當有限，幾近沒有影響。

4-3 RO 純水添加深層海水濃縮液之後對導電度之影響

將深層海水濃縮液加入 RO 純水中，僅需 0.05ml(1 滴)即可將 500ml 之 RO 純水導電度由 10 提升至 120 μs/cm，有效改善 RO 純水之導電度過低之問題，更進而改善 RO 純水無法與電解水機配合進行電解水之製造問題。

5. 結論

由包裝水之導電度量測可得知，多款知名品牌包裝水應是以 RO 裝置加以進行飲用水之前處理。故而 RO 裝置仍是目前飲用水處理之主流。活性碳之添加證實可有效提升飲用水之導電度。深層海水濃縮液之添加亦證實可有效提升 RO 純水之導電度。

P15. Packaged drinking water conductivity analysis and reversed osmosis improvement of drinking water quality by deep sea water concentrate

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1. Introduction

Drinking water treatment technology is developed to obtain high-quality and safe drinking water for humans. Drinking water for humans is very important. Therefore, the ancients had a saying "Water is king of drugs."

Drinking water treatment technology was developed from the early traditional physical filtration process to the precision filtration of polymer film filtering procedure. With the industrial development and the increase of population density in metropolitan areas, raw water quality is deteriorating rapidly with industrial and livestock pollution. Although tap water companies provided water according to the drinking water standards, there are still many problems cannot be solved. People are still seeking various high-tech procedures to ensure the safety of their drinking water. Buying packaged drinking water and the set up of reversed osmosis (RO) purification equipment are the most common way of drinking water supply in recent years.

The advantages of packaged drinking water are the convenience of purchase and the security assurance of drinking water providers.The disadvantage of drinking packaging water is heavier burden on people's economy. Most people choose to believe the manufacturers, or based on price. There are few literature about the evaluation of packaged drinking water,hence, this study.

The packaged drinking water are named mineral water, pure water, ocean generated water, deep sea water; or distilled water. The conductivity, which is the easiest way to indicate the mimeral content, was not included in this study as we measured the conductivity of packaged drinking water using a conductivity meter.

2. Experimental methods

2-1. Measurement of conductivity and pH

Each 5 ml samples were placed into 30 ml sample containers. Put the electode of conductivity meter and pH meter into the content, respectively, to measure the value.

2-2. Effect of activated carbon and medical stone addition on the RO pure water conductivity.

The fine scale of activated carbon or medical stone was put into the beaker of 100 ml RO water and then placed on the magnetic stirrer.The conductivity values were measured during fixed time interval.

2-3. Effect of deep sea water concentrate addition on the RO pure water conductivity.

Successive deep sea water concentrate was added into a beaker with 500 ml pure RO and conductivity was measured.

3. Experimetal material.

Ocean-generated water (Taiyen Company), healthy pure water (standard beverage company), LOHAS advocate (Coca-Cola), yes (Young Energy Source Co., Ltd.(YES)), Alkaline ion water (Taiyen Company)

4. Results and Discussion

4-1. Comparsion of conductivity of packaged drinking water, RO pure water and distilled water.

	Conductivity(μs/cm)	PH
(1)Distilled water	1.43	6.56
(2)RO pure water	6.33	6.23
(3) Atlantic water	1.86	7.82
(4) 100 years good water	4.07	6.27
(5) Healthy pure water	10.27	8.71
(6) LOHAS advocate	25.7	7.53
(7) Yes	38	7.49
(8) Ocean-generated water	42.2	8.48
(9) Boer mineral water	153.8	7.43
(10)Drinking more water	247	7.1
(11) Charcoal water	266	7.46
(12) Deep vitality	336	6.12

4-2. Effect of activated carbon and medical stone addition on the RO pure water conductivity.

RO water retention in the activated carbon can effectively improve the RO water conductivity values. RO water retention in the medical stone showed almost no effect and the value of the conductivity was very limited.

4-3.Effect of deep sea water concentrate addition on the RO pure water conductivity.

When the deep sea water concentrate was added to 500 ml RO water, only 0.05 ml (1 drop) improved the conductivity of RO pure water from 10 to 120 μs/cm, and the conductivity of the RO pure water improved. This improvement also overcome the problem since RO pure water cannot combine with electrolysis water machine to manifacture ionized water.

5. Conclusion

Conductivity measurement of packaged drinking water on a variety of well-known bottled water brands should be RO unit for drinking water pretreatment process. Therefore RO drinking water treatment device is still the main stream. Activated carbon addition can enhance the conductivity of water. Deep sea water concentrate additions can effectively enhance the conductivity of RO pure water.

P16. 深層海水啤酒之感官特性研究

林雪良(台北海洋技術學院)、°陳盈貝、黃秉益(石資中心)、任曉晶(台北海洋技術學院)

1. 前言

台灣深層海水取自太平洋海平面 600 公尺下，免於地表有害物質及病原菌、難分解性有機汙染物等汙染，且深層海水長年處於高壓、低溫環境，水質潔淨又穩定。深層海水含有多種營養鹽及微量元素，其礦物質組成與人體細胞相近，是台灣東部特有的珍貴水資源。

本研究以台灣花蓮深層海水原料添加於啤酒水體中進行釀造，探討利用深層海水之多元礦物質於啤酒釀製各階段所引起的變化，找尋最佳之條件，以作為酒類應用之基礎。

2.材料和方法

本研究目的針對添加不同濃度深層海水原料之大麥及螺旋藻口味啤酒，以未加深層海水為對照組，利用感官品評試驗和成分分析結合多變量統計方法，了解產品成分、特性及消費者喜好程度。

深層海水啤酒感官品評方法

研究人員針對 80 位消費者品評員在宜蘭礁溪進行大麥及螺旋藻啤酒消費者接受性 (九分制) 與喜好性 (排序法) 試驗，並於品評結束後進行調查。深層水啤酒共分成為七組進行消費者品評試驗, 研究年齡層為 18 歲以上且接受啤酒之消費者，實驗結果以變異數分析與多變量統計進行分析。

深層海水啤酒之成分分析

添加深層海水濃縮礦物質液調整啤酒釀造水體硬度為 100 ppm 或 300 ppm，並分析啤酒 pH 值、糖度、酒精度、酸度、色度、苦味值、褐變程度及二氧化硫等，了解啤酒品質影響。

3.結果和討論

研究發現添加深層海水之大麥及螺旋藻啤酒之 pH 值 4.6~5.03，總糖含量為 4.2~4.8 g/100 mL，酒精度範圍為 4.0~5.3 %vol，螺旋藻啤酒 Hunter a 值範圍為 10.9~21.7，其綠色度以硬度 300 ppm 最佳。添加不同比例深層海水之大麥啤酒進行熟成後，發現添加硬度濃度愈高啤酒之褐變程度較低，獲得大麥和螺旋藻啤酒之基本成分差異。

消費者喜好性品評結果顯示，嚐味性為影響消費者喜好深層海水啤酒之主要特性。依九分制品評單所評定之品質優良的啤酒亦符合消費者的喜好，故此九分制品評單能做為一套深層海水啤酒感官品質評估的標準。實驗結果顯示深層海水之添加與否在啤酒香氣上並無影響；嚐味性及外觀則以添加深層水之組別表現較佳。經品評試驗，可得知深層海水啤酒的香氣、顏色、風味、總評，和商業啤酒相同，證實使用深層海水原料於啤酒釀造有應用之價值。

P16. Studies on sensory attributes in deep sea-water beer

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1. Introduction

Deep sea-water (DSW) near Taiwan is pumped up from 600 m below sea level in the Pacific Ocean. This water is free of pollutants, such as harmful substances on the earth surface, pathogenic bacteria, and persistent organic pollutants. In addition, because DSW is under high pressure in a low-temperature environment for a prolonged period, the seawater possesses clean and stable water quality. Furthermore, the water contains various nutrient salts and trace elements, whose mineral compositions are similar to that of human cells. Consequently, this seawater is considered a valuable resource that is found exclusively in Eastern Taiwan.

We added DSW, obtained off the coast of Hualien, Taiwan to the beer-brewing process. The objective was to use the multi-mineral characteristics of DSW to explore the changes induced during various stages of the beer-brewing process to determine optimal beer-brewing conditions and develop alcoholic products that are unique to Taiwan.

2. Materials and Methods

Barley and *Spirulina* beers brewed using varying DSW concentrations were set as the experimental group and beer without DSW was set as the control group. Sensory-evaluation tests and composition analyses were combined using multivariate statistics to evaluate the product ingredients, characteristics, and consumer preferences.

2.1 Deep Sea-water beer sensory-evaluation methods

Overall, 80 consumers were recruited to serve as the sensory-evaluation subjects for conducting an acceptance test (using a 9-point hedonic scale) and preference test (by ranking) on barley and *Spirulina* beers in Jiaoxi, Yilan. Analyses were performed after the sensory evaluations were completed. Overall, 7 experimental groups comprising the subjects were instructed to perform the sensory evaluation test. The consumers of study were all over 18 years of age and had provided their consent to participate in the test. The results were subsequently analyzed using analysis of variance (ANOVA) and multivariate statistics.

2.2 Deep sea-water beer composition analysis

Solutions of concentrated liquid minerals obtained from DSW were added to alter the hardness of the water (100 or 300 ppm) used for brewing beer. The beer pH value, sugar content (in degrees Plato), alcohol, titratable acidity, chromaticity, bitterness, and sulfur dioxide were analyzed to determine the influences on the beer quality.

3. Results and Discussion

The results showed that after adding the concentrated liquid minerals, the pH value of barley and *Spirulina* beers was between 4.63 and 5.0, the sugar content was between 4.20 and 4.73°P, and 5% (V/V) ethanol was produced. It showed that when the higher concentration of liquid minerals was added, the chromaticity of the barley beer decreased. However, the green hue in chromaticity increased in *Spirulina* beer,. It also showed that when a higher concentration of liquid minerals was added, the less bitterness of beers was observed. Sulfur dioxide was not detected in any of the groups. The results presented the differences of the basic components between barley and *Spirulina* beers. The subject sensory-evaluation results indicated that, although the barley beer contained a water hardness of 100 ppm, no significant differences were exhibited compared with the control group regarding taste, mouth-feel, and overall flavor preference. However, the mean sensory-evaluation scores were all higher than those of the control group. This indicated that the quality of barley beer is enhanced by adding sufficient amounts of concentrated liquid minerals, which warrants further study. The *Spirulina* beer-test results showed that beer containing 300-ppm water hardness demonstrated a significant difference from beer containing 100-ppm hardness in appearance and aroma, signifying that adding DSW-concentrated liquid minerals improves the appearance and aroma of *Spirulina* beer. This can enhance product competitiveness.

It is concluded that adding DSW concentrated liquid minerals enhances beer-brewing quality. It is feasible to use DSW when brewing beer.

P17. 深層海水礦物質濃縮液之製程探討

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1.前言：

深層海水資源的產業利用中，濃縮礦物質、養殖及冷能是主要應用標的。其中礦物質濃縮之核心技術主要是在分離海水中之無用物質如鈉離子、氯離子與硫酸根離子，並保留有用物質如鈣離子與鎂離子。在先進國家日本主要是利用電透析設備(ED)來去除硫酸根與鈉離子，而台灣則因設備成本及運轉成本的因素，以利用減壓蒸餾濃縮設備所製造之礦物質液為主要的應用原料，但其規格變化性低，間接影響後端產品之多樣化。因此，本中心積極投入利用奈米膜(NF)特性，再搭配減壓蒸餾濃縮製程之方式，研發可接近甚至超越ED規格之礦物質液之生產技術。本研究將探討在 NF 與減壓濃縮設備之操作條件下對礦物質濃縮液規格之影響。

2.方法：

礦物質生產系統由 2 套 NF 與 1 套逆 滲透過濾(RO)及 1 套減壓濃縮設備所構成。NF1 主要功能為提濃 Ca^{2+}、Mg^{2+}離子，NF2 為脫除硫酸根離子，原水處理量分別為 50ton/batch 及 100ton/batch。低溫減壓濃縮設備為七福工業製，處理量為 2.4ton/day。本研究利用取自台東知本外海之深層海水原水經 NF1、NF2 之處理後，獲得初級礦物質液，再以不同之條件測試減壓蒸餾最佳操作參數。

3.結果與討論：

經 NF1、NF2 提濃處理後得到之 DSW 礦物質液之主要離子含量為 Ca^{2+}：220 mg/L、Mg^{2+}：380 mg/L、Na^{+}：6,620mg/L，SO_4^{2-}=120mg/L，Ca/Mg 比為 0.6，硬度約為 2,108ppm，將此產物導入 RO 提濃後，投入減壓濃縮設備。在濃縮製程進行時，隨著加熱時間的增加，產品硬度隨之增高，在不同加熱時間取樣分析的結果發現，產品鹽度與硬度變化成正向關係。在鹽度為 30‰以下時，硬度對鹽度之變化斜率較為平緩，但在鹽度增加至 30‰以上時，硬度有指數性增加的趨勢。當產品硬度小於 20,000mg/L 時，鈉離子濃度與硬度成正比增加，在硬度達 20,000mg/L 時，鈉離子濃度達最大值(≒110,000mg/L)後，開始下降，而鎂離子、鈣離子及氯離子濃度則開始上升。當產品硬度到達 60,000mg/L 時，硫酸根離子濃度呈最大值後開始下降。此時濃度積 $[Ca^{2+}][SO4^{2-}]=1.74x10^{-3} >> CaSO_4$ 之 $k_{sp}=5x10^{-5}$，因大量之硫酸鈣析出，使得溶液中硫酸根離子濃度下降。減壓蒸餾過程發現，當真空度與操作溫度之飽和蒸汽壓相當時，反應器內部會有突沸現象，導致部分礦物質濃縮液被真空泵抽出，使的產量銳減。故為求產物之穩定產出，必須小心調整蒸餾時之溫度，將有助於產品之品質與產出量。

P17. Producing mineral concentrates from deep sea water

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Water Resource Group, Stone and Resource Industry R&D Center, Hualien, Taiwan

1. Introduction:

The deep sea water (DSW) industry can be roughly divided into the following branches: (1) mineral concentration; (2) farming; and (3) cold energy use. Mineral concentration technology is used to reduce the concentrations of unwanted substances, such as sodium, sulfate, and chloride ions, and maintain the quantities of useful substances such as calcium and magnesium ions. In advanced countries including Japan, electric dialysis (ED) is used to remove sulfate and sodium ions. In Taiwan, because of high equipment and operating costs, the vacuum distillation process has typically been used to produce mineral concentrates of raw materials. However, such raw materials have few specifications, indirectly limiting the variety of final products. Accordingly, the Stone and Resource Industry R&D Center (SRDC) uses the nanofiltration (NF) collocation vacuum distillation process to produce mineral concentrates and investigate mineral concentrate production technologies comparable to the ED specification. In this study, we explored the operating conditions used in the NF collocation vacuum distillation process, determining how these conditions affect the specifications of mineral concentrates.

2. Materials and Methods

The mineral concentrate production system comprised two sets of NF, one set of reversed osmosis (RO), and vacuum distillation equipment (Lucky Seven Industrial). The NF1 was used to concentrate Ca^{2+} and Mg^{2+} ions and the NF2 was used to reduce the Ca^{2+} and, Mg^{2+} ions. DSW was fed at 50 ton/batch and 100 ton/batch. The feed used in the vacuum distillation process was 2.4 ton/d. The DSW was sourced from the ocean located at Taimali Township, Taitung, Taiwan. The DSW was treated using NF1 and NF2 to obtain primary mineral concentrates. Moreover, the operational parameters were set to various testing conditions.

3. Results and Discussion

After the NF1 and NF2 treatments, the primary mineral ion concentrations of the effluent were Ca^{2+} (220 mg/L), Mg^{2+} (380 mg/L), Na^{+} (6620 mg/L), and SO_4^{2-} (120 mg/L) . The ratio of Ca/Mg was 0.6 and the hardness was 2108 mg/L. Moreover, the effluent was fed to the RO for concentration and then fed to the vacuum distillation equipment. The hardness of the intermedium was gradually increased during the distillation process. The intermedium was sampled to analyze the composition at various heating times. The hardness and salinity variations were positively correlated. When the salinity was below 30%, the hardness slope was gentle; however, the hardness slope exponentially increased when salinity exceeded 30%. The sodium ion concentration increased as the hardness increased when the hardness was below 20000 mg/L, reaching a maximal value (≒110000 mg/L), then decreasing as the hardness increased when the hardness surpassed 20000 mg/L. The magnesium, calcium, and chloride ion levels increased as hardness increased. The sulfate ion concentration increased as hardness increased when the hardness was below 60000 mg/L, reaching a maximal value, then decreasing as hardness increased when the hardness exceeded 60 000mg/L. At this time, the multiplication of $[Ca^{2+}]$ $[SO_4^{2-}]$(= $1.74x10^{-3}$) was larger than was that of the k_{sp}(= 5×10^{-5}). Therefore, the $CaSO_4$ crystallized, causing the sulfate ions to decrease as the hardness increased. During the vacuum distillation process, sudden boiling occurred when the degree of the vacuum was equal to the vapor saturate pressure of the temperature condition. Therefore, part of the mineral concentrate was extracted using the vacuum pump, reducing the mineral yield. Therefore, carefully controlling the distillation temperature improves the product quantity and yield.

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民國79年》
為平衡東西部經濟發展，振興傳統產業，財團法人石材工業發展中心於民國81年8月成立籌備會，由經濟部工業局、台灣區石礦製品工業同業公會及石材業共同捐助基金4千萬元成立。

民國95年》
因應東部產業轉型升級，以及單位永續發展，經董監事會同意後，於同年5月正式更名為「財團法人石材暨資源產業研究發展中心」並擴充軟、硬體設施以既有石材相關，技術能量為基礎，積極研發深層海水應用技術肇基「藍金」產業。

民國98年》
配合政府各項政策推動，本中心新增東部產業技術服務中心、東區創業創新服務中心及因應ECFA之產業輔導單位等多重任務，加上多年投入與經營東部地方特色產業輔導開花結果，擴充研發人力與經費於地方特色產業的創新服務與關懷輔導，並成立專責部門積極推動，期許本中心成為「東部地區的創新服務平台」，對於東部地區的產業發展將扮演重要的推手角色。

經濟部東部深層海水創新研發中心
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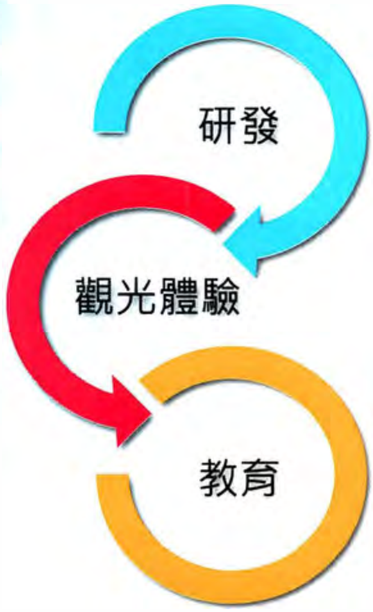

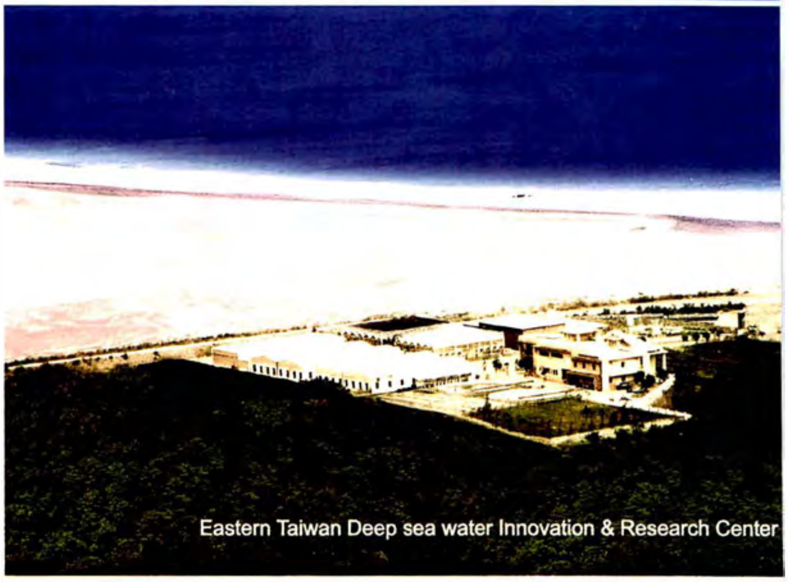

簡介
經濟部東部深層海水創新研發中心，由水利署發包興建，101年5月正式移交予經濟部技術處營運，初期營運方向為，運用深層海水特性，開發高價值應用產品，帶動產業創新發展。結合產學界資源，建立東部研發資源整合與服務分享平台，支持東部特色產業創新與發展。扶植東部新創事業，帶動東部特色／新興產業發展。後續並將以技術研發開發能量為基礎打造兼具多元教育體驗功能及觀光休憩示範空間，帶領台灣走向藍金產業的新領域。

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本會宗旨

本會為依法設立、非營利為目地之社會團體，以配合中央政府推動「深層海水資源利用及產業發展綱領計畫」，結合海洋深層水相關新興商品之技術研發、製造和商業服務創新行銷等各領域資源和人力，建構成實體合作社群，共同進行價值創新，發展台灣東部深層海水資源成高附加價值產業為設立宗旨。

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(五)、有關協助業者辦理國內外展覽、會議之推廣事項。
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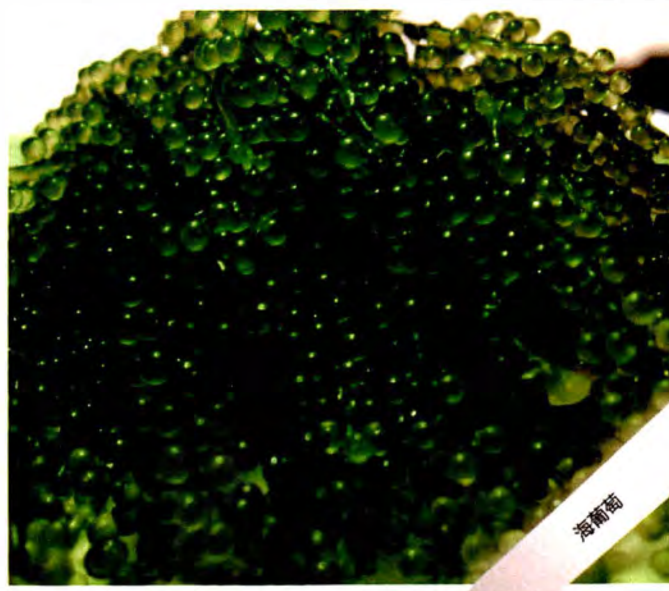
台灣花蓮太平洋一662公尺深之海洋深層水，經日本專家技術合作培育、養殖、養生、製作。

天然　營養　美食
海葡萄是屬總狀蕨藻類的海藻，生長在熱帶及亞熱帶地區零污染的水域中，它外觀像一串晶瑩碧綠的葡萄一樣;海葡萄含有豐富的膳食纖維，各種礦物質及人體需要的多醣體等等。具有低卡路里及無膽固醇的實用特性，咬下去的口感有如新鮮魚子醬一樣，其鹹鹹的汁液帶有淡淡海洋的氣息，就因為海葡萄有著魚子醬的口感，故又被喻為綠色魚子醬。海葡萄有著天然植物所擁有對人體機能需要的成分，這些對現代人的諸多文明病有著預防及抑制的效果。海葡萄提供最天然及原始的物質給人體，使人體活出健康與活力。



海藻的成分

海藻中的胺基酸及脂肪酸：
具有控制血壓降低高血壓患者發生中風之風險、降低膽固醇、防止血栓形成及心肌梗塞等，對心血管疾病有預防作用。

海藻中的礦物質：
生長在海水裡的海藻其擁有比陸地上植物更多、更天然的礦物質，海藻中之礦物質又以鈉、鉀、鐵、鈣、鎂含量最多。人類若缺乏上述元素時身體機能就會失衡，所以平日多攝取海藻以補充無機元素。

海藻中的維生素：
海藻含有多種維他命，主要以維生素B群為主，也含維生素C、E及菸鹼酸等，可預防人體新陳代謝失調等疾病的發生。

海藻中的膳食纖維：
食物纖維是具有多醣類結構的大分子，能幫助腸道排出致癌之有害物質，改善高血脂症狀、抑制膽固醇增加、預防及改善糖尿病患者對血糖量之控制。

海藻中的多醣體：
海藻具有增強免疫力及抗癌活性的物質，海藻中的褐藻醣膠，經研究發現具啟動癌細胞凋亡機制。

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從發現礦物質開始
1天2瓶・完整均衡
補充人體所需之礦物質微量元素
■每瓶含鎂(200毫克以上)、鉀、鈉、鈣等多種天然離子態礦物質微量元素，完整均衡容易吸收利用。
■礦物質微量元素是人體必需營養素，能調節生理機能，健康維持。鎂參與醣類和能量代謝，與鈣共同維持心臟、肌肉及神經等正常功能。
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