

이학석사학위논문

2006-2008 년 독도 주변 해역에서 미생물 상호 관계 및  
분리된 2 개의 신종 박테리아 연구

Study on characteristics of the microbial interrelationships and two novel  
bacteria isolated in the vicinity of Dokdo in 2006-2008

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## 영문초록

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To understand the characteristics of the microbial interrelationships and to isolate and identify novel bacteria in the vicinity of Dokdo, investigations were performed in the upper 100 m depth in North and South transects of the Dokdo island in Nov. 2006, Mar. 2007, Dec. 2007 and Mar. 2008.

During the first investigation (in Nov. 2006), physical and chemical properties of water column in North and South of the Dokdo were different each other, probably owing to more active vertical mixing in South than in North of Dokdo (consistent with the facts that deeper mixed-layer and higher nitrate concentration at the surface were observed in South than those in North of the Dokdo). In this investigation, viral (VA) and heterotrophic nanoflagellate abundance (HNFA) in the mixed layer of South were 1.3- and 1.4-fold higher than those in North, respectively ( $t$ -test,  $p < 0.05$ ). However, positive correlations between bacterial abundance (BA) and bacterial production based on  $^{14}\text{C}$ -leucine incorporation (BP-Leu;  $r^2 = 0.73$ ,  $p < 0.05$ ,  $n = 8$ ), and between BA and HNFA ( $r^2 = 0.27$ ,  $p < 0.05$ ,  $n = 15$ ) were significant in North, but not in South.

During the second investigation (in Mar. 2007), the difference of

physical properties of water columns shown in the first investigation was obviously decreased between South and North transects. No significant differences in BA, VA and HNFA were observed between both areas. In addition, correlations of most variables showed similar results between North and South transects, except for some correlations (e.g. a positive correlation between BA and HNFA was significant only in North).

During the third and forth investigations, physical properties of water columns of North and South seas were similar, and abundances and interrelationships of microbial variables were also similar.

During this study, ca. 200 strains were isolated using a plate culture method. Subsequently, 84 strains were randomly selected and analyzed with 16S rRNA gene sequences. In the examined strains, 10 strains (11.9% of the examined strains) showed less than 97% similarity to that of the validly published species based on 16S rRNA gene sequences, suggesting that those 10 strains could be novel species. Two strains were further studied and found to be new species in the genera *Nocardioides* and *Arcobacter* using polyphasic taxonomic analyses.

It was shown that microbial interrelationships might be controlled by changes of physical and chemical properties in the vicinity of Dokdo. And the correlations of microbial variables obtained in this study were similar to those from studies which were investigated in open ocean, suggesting that the vicinity of Dokdo is a scientifically interesting area in oceanic researches. Furthermore, the vicinity of Dokdo needs to be preserved for its value of microbial resource.

Keywords: Dokdo, microbial interrelationships, bacteria, virus,  
heterotrophic nanoflagellate, novel bacteria

Student number: 2006-20457



## 포텐셜 자료를 이용한 울릉분지와 독도 주변 지체구조 연구

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## Tectonic Structure Modeling around the Ulleung Basin and Dokdo Using Potential Data

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**Abstract:** The East Sea including the area of this study is identified as a typical back-arc sea located in the backside of the Circum-Pacific volcanic and earthquake belt. Previous studies reported that the East Sea has begun to open by tensile force and formed its current shape. In this study, we investigate the regional tectonic structure of the East Sea using ship-borne gravity, magnetic, and satellite gravity data. The result of three-dimensional depth inversion shows that Moho depth of the study area is approximately 13-25 km and inversely proportional to the thickness of the crust. In addition, as approaching to the center of the Ulleung Basin (UB), the thickness of the crust of the UB becomes thinner due to the extension caused by tensile force which had opened the East Sea.

**Keywords:** Ulleung Basin, Potential data, Moho discontinuity

**요약:** 연구지역을 포함하는 동해는 환태평양 화산 및 지진대 뒤편에 형성된 전형적인 후열도해이다. 기존 연구에 의하면 동해는 인장력을 받아 열리기 시작하여 현재 동해의 모습을 갖추었다고 알려져 있다. 이번 연구에서는 이러한 동해의 지체구조 특성을 연구하기 위하여 선상에서 측정된 중·자력 자료 및 인공위성 중력 자료를 이용하여 울릉분지와 독도 주변 동해 지역의 지체구조 연구를 수행하였다. 3차원 심도역산 결과, 연구지역의 모호면은 13-25 km 정도의 심도를 갖으며 지각의 두께와 반비례하는 경향을 보여주었다. 또한, 동해의 확장과 함께 생성된 울릉분지는 인장력으로 인해 그 중심으로 갈수록 지각 두께가 감소하는 특성을 확인할 수 있었다.

**주요어:** 울릉분지, 포텐셜, 모호면

## 서론

동해는 지리적으로 한국, 일본, 러시아에 의해 둘러싸인 반폐쇄형 바다로서 중심 지역의 수심이 2000 m가 넘는 대양의 성격을 갖는다. 판구조론의 관점에서는 유라시아판, 태평양판, 필리핀판 등으로 경계

지어지고, 일본 열도에 의해 태평양과 분리되어 유라시아 판내에 위치한다. 동해는 울릉분지, 일본분지, 그리고 야마토분지를 포함하고 있으며, 이들 분지는 해령과 해저대지들로 구분되어 독립적으로 발달해 있다(Jolivet et al., 1991). 이러한 분지들의 형성 과정을 이해하기 위하여 동해 성인에 관한 연구가 꾸준히 진행되고 있다. 동해 성인에 관하여 학자들마다 다소 상이한 견해를 보이고 있는데, 울릉분지를 포함한 동해의 분지들은 후열도분지(back-arc basin)이고, 이 중 울릉분지와 야마토분지가 대륙지각인지 해양지

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## Vertical Structure of Low-Frequency Currents in the Southwestern East Sea (Sea of Japan)

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The vertical structure of low-frequency flows in the central Ulleung Interplain Gap of the southwestern East Sea (Sea of Japan) is analyzed based on full-depth current measurement during November 2002–April 2004. Record-length mean flows are directed toward the Ulleung Basin (Tsushima Basin) throughout the entire water column. Upper current variability above the permanent thermocline with a dominant period of about 50–60 days is shown to be closely related to the displacement of an anticyclonic warm eddy associated with the westward meander of the Offshore Branch. Fluctuations of deep currents below the permanent thermocline have a dominant period of about 40 days. Coherence between the current near the seabed and shallower depths is statistically significant up to 360 m for a period range between 15 and 100 days, but less significantly correlated with currents in the upper 200 m. Data from the densely equipped mooring line reveal that mean and eddy kinetic energies are minima at 1000 m, where isotherm slopes are also relatively flat. Empirical orthogonal function (EOF) analyses suggest that more than 79% of total variances of upper and deep currents can be explained by their respective first EOF mode characterized by nearly depth-independent eigenvectors. Spectral and EOF analyses of observed currents suggest that most of the deep current variability is not directly related to local upper current variability during the observation period.

Keywords:

- Sea of Japan,
- moored current observation,
- low-frequency current variability,
- vertical structure of current.

### 1. Introduction

The East Sea<sup>§</sup> is a semi-enclosed marginal sea in the northwestern Pacific. The sea consists of three basins deeper than 2000 m: the Japan Basin (JB), the Ulleung Basin<sup>¶</sup> (UB), and the Yamato Basin (Fig. 1). The Ulleung Interplain Gap<sup>†</sup> (UIG), located in the southwestern East Sea, is approximately 90 km wide, and is a nearly symmetric, deep channel with a maximum depth of approximately 2300 m at its southwestern tip. Deep water is formed in the JB (Kim *et al.*, 2004), leading to a thermohaline circulation that transports deep, cold water

toward the southern basins. The Korea Plateau, which is shallower than 1500 m, lies between the JB and UB, whereas the Oki Spur, which is shallower than 500 m, acts as a barrier to deep water exchange between the UB and Yamato Basin. Thus, the UIG serves as a passageway for the exchange of deep waters below 1500 m between the UB and the JB (Kim *et al.*, 1991). Direct measurement of currents through the UIG would provide useful information to help us understand the thermohaline circulation of the East Sea and deep circulation in the UB.

The Subpolar Front (SPF), which is a strong thermal boundary in the central part of the East Sea, separates the southern warm water region from the northern cold water region. Mitchell *et al.* (2005b) reported that the SPF frequently forms a large meander trough within the UIG and oscillates in an east-west direction with a period of approximately 60 days. The SPF meander allows cold water north of the SPF to be brought into the UB. Upper circulation in the UB south of the SPF is affected mainly

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<sup>§</sup> The Editor-in-Chief does not recommend the usage of the term “East Sea” in place of “Sea of Japan” or “Japan Sea”.

<sup>¶</sup> The Editor-in-Chief does not recommend the usage of the term “Ulleung Basin” in place of “Tsushima Basin”.

<sup>†</sup> The “Ulleung Interplain Gap” corresponds to the “Oki Gap”.

## *Pelagibius litoralis* gen. nov., sp. nov., a marine bacterium in the family *Rhodospirillaceae* isolated from coastal seawater

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A Gram-negative, strictly aerobic, slightly curved rod-shaped bacterial strain, designated CL-UU02<sup>T</sup>, was isolated from coastal seawater off the east coast of Korea. 16S rRNA gene sequence analysis revealed a clear affiliation of this novel strain with the family *Rhodospirillaceae*. Strain CL-UU02<sup>T</sup> formed a robust cluster with the type strains of species of the genus *Rhodovibrio* at 16S rRNA gene sequence similarity levels of 89.9–90.4 %. Strain CL-UU02<sup>T</sup> shared no more than 89 % 16S rRNA gene sequence similarity with the type strains of other species in the family *Rhodospirillaceae*. Strain CL-UU02<sup>T</sup> was able to grow in the presence of 2–6 % sea salts, and grew optimally at 28–30 °C and pH 7–8. The DNA G + C content of strain CL-UU02<sup>T</sup> was 66.3 mol%. On the basis of phylogenetic analyses and chemotaxonomic and physiological data, strain CL-UU02<sup>T</sup> is considered to represent a novel species of a new genus in the family *Rhodospirillaceae*, for which the name *Pelagibius litoralis* gen. nov., sp. nov. is proposed. The type strain of *Pelagibius litoralis* is CL-UU02<sup>T</sup> (=KCCM 42323<sup>T</sup>=JCM 15426<sup>T</sup>).

The order *Rhodospirillales* currently comprises two families, *Rhodospirillaceae* and *Acetobacteraceae*, in the class *Alphaproteobacteria* (Garrrity *et al.*, 2005). At the time of writing, the family *Rhodospirillaceae* comprises 16 genera, namely *Azospirillum*, *Caenispirillum*, *Defluviicoccus*, *Inquilinus*, *Magnetospirillum*, *Phaeospirillum*, *Rhodocista*, *Rhodospira*, *Rhodospirillum*, *Rhodovibrio*, *Roseospira*, *Skermanella*, *Telmatospirillum*, *Thalassobaculum*, *Thalassospira* and *Tistrella* (see <http://www.bacterio.cict.fr>).

Among 40 recognized species in the family *Rhodospirillaceae*, only seven species affiliated with the genera *Rhodovibrio* (Mack *et al.*, 1993), *Rhodospira* (Pfennig *et al.*, 1997), *Thalassospira* (López-López *et al.*, 2002; Liu *et al.*, 2007; Kodama *et al.*, 2008) and *Thalassobaculum* (Zhang *et al.*, 2008) have been recovered from marine environments. Other species in the family *Rhodospirillaceae* have been isolated from various non-marine habitats, such as freshwater, activated sludge biomass, air, soil and roots of plants, and cystic fibrosis patients (Coenye *et al.*, 2002; Garrrity *et al.*, 2005; Weon *et al.*, 2007; Yoon *et al.*, 2007). In the present study, a novel

bacterial strain, designated CL-UU02<sup>T</sup>, affiliated with the family *Rhodospirillaceae* was isolated from urea-enriched seawater and was subjected to a polyphasic taxonomic analysis.

In February 2005, coastal seawater taken from the east coast of Korea was brought back to the laboratory for analysis. One hundred microlitres of seawater was inoculated in autoclaved seawater (500 ml) supplemented with urea (final concentration of 100 mM) and incubated at 20 °C in the dark. After about 8 months, 100 µl of the sample was taken and spread on a marine agar 2216 (MA; Difco) plate, which was then incubated aerobically at 30 °C for 2 weeks. Strain CL-UU02<sup>T</sup> was isolated and subsequently streaked onto fresh MA plates at 30 °C under aerobic conditions. The purification procedure was repeated four times. Strain CL-UU02<sup>T</sup> was maintained both on MA at 30 °C and in marine broth 2216 (MB; Difco) supplemented with 30 % (v/v) glycerol at –80 °C.

For 16S rRNA gene amplification by PCR, DNA was extracted from a single colony based on a boiling method (Englen & Kelley, 2000). The crude extracts served as the DNA template for PCRs, which included *Taq* DNA polymerase (Bioneer) and primers 27F and 1492R (Lane, 1991). The PCR product was purified by using an AccuPrep PCR purification kit (Bioneer) and was cloned by using pGEM T-Easy vector (Promega). Sequencing of

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain CL-UU02<sup>T</sup> is DQ401091.

An extended neighbour-joining tree based on 16S rRNA gene sequences showing the position of strain CL-UU02<sup>T</sup> among members of the family *Rhodospirillaceae* is available as supplementary material with the online version of this paper.

## Note

**북서태평양에서 난수성(*Pleuromamma* sp.)과 냉수성(*Neocalanus plumchrus*)  
요각류의 지방 함량 및 구성 분석을 통한 생리/생태 비교**고아라<sup>1,2</sup> · 주세종<sup>2\*</sup> · 이창래<sup>2</sup><sup>1</sup>한양대학교 과학기술학부 해양환경과학과  
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(425-600) 경기도 안산시 안산우체국 사서함 29**The Physiological and Ecological Comparisons between Warm  
(*Pleuromamma* sp.) and Cold Water Copepod Species  
(*Neocalanus plumchrus*) in the Northwestern Pacific  
Ocean Using Lipid Contents and Compositions**Ah-Ra Ko<sup>1,2</sup>, Se-Jong Ju<sup>2\*</sup>, and Chang-Rae Lee<sup>2</sup><sup>1</sup>Department of Environmental Marine Sciences Division of Science and Technology  
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**Abstract :** In an effort to better understand the physiological and ecological differences between warm and cold water copepod species in Korean waters using lipid contents and compositions, two species of copepods (*Pleuromamma* sp. as a warm water species and *Neocalanus plumchrus* as a cold water species) were collected from the Northwest Pacific and East Sea/Sea of Japan, respectively. The cold water species showed two fold higher lipid contents than the warm water species (11% vs. 5% of dry weight). Wax esters, known as one of the major storage lipid classes, were found to be the dominant lipid class (accounting for 64% of total lipids) in the cold water species, whereas, in the warm water species, phospholipids, which are known as membrane components, were the dominant lipid class (accounting for 43% of total lipids), with a trace amount of the storage lipids as a form of triacylglycerols ( $\leq 1\%$  of total lipids). With regard to the fatty acid compositions, saturated fatty acids (SAFA), especially 16:0 (about 30% of total fatty acids), were most abundant in the warm water species, whereas the polyunsaturated fatty acids (PUFA), particularly eicosapentaenoic acid (EPA : 20:5(n-3)) ( $\geq 16\%$  of total fatty acids), were most abundant in the cold water species. Among the neutral fraction of lipids, phytol, originating from the side chain of chlorophyll and indicative of active feeding on phytoplankton, was detected only in the warm water species. Significant quantities of fatty alcohols were detected in cold water species, particularly long-chain monounsaturated fatty alcohols (i.e. 20:1(n-9) and 22:1(n-11)), which are well known to abound in cold water herbivorous copepods. However, only trace amounts of short-chain fatty alcohols were detected in the warm water species. Twelve different kinds of sterols were detected in these copepod species, with cholest-5-en-3 $\beta$ -ol (cholesterol) and cholest-5, 24-dien-3 $\beta$ -ol (desmosterol) dominating in cold and warm water species, respectively. In addition, for the warm water species (*Pleuromamma* sp.), we assessed the latitudinal gradients of lipid contents and compositions using samples from three different latitudinal regions

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기술보고

# 해수 중의 미량금속 분석을 위한 청결기술의 소개

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## Introduction of Clean Techniques for Trace Metal Analysis in Seawater

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**요 약 :** Al, Ag, Au, Cu, Cd, Co, Fe, Ni, Pb, Zn 등의 금속은 해수 중에 미량으로 존재하기 때문에 미량금속이라고 하며 일부는 미량영양소로서 생명체의 생리활동에 필요하지만 정해진 한계값을 초과할 때는 독성을 나타낸다. 미량금속(중금속)은 해양환경 및 해양생태계에 악영향을 미칠 수 있기 때문에 지속성 오염물질로 분류되어 다양한 연구가 수행되고 있다. 해수와 담수 등 자연수 중의 미량금속 측정은 정확도와 정밀도에 있어서 큰 오차를 가지고 있음이 장기간 인식되어 왔다. 미국과 유럽에서는 1975년 이후 미량금속의 해수 중 농도가 과거에 인지된 농도의 10~1/1,000 정도로 낮아졌으며, 수직 분포는 생물, 물리, 지화화학적 과정들이 반영되고 있음이 밝혀졌다. 이와 같은 결과는 대부분 미량금속에 대한 분석방법과 기기의 발전에 기인하며, 시료 채취, 보관 및 분석 과정에 발생할 수 있는 오염을 제거해야 하는 세심한 주의가 요구되고 있다. 그러나 국내의 경우 많은 해양환경 관련 조사 및 연구에서 정확성이 결여된 자료가 보고되고 있다. 특히 미량금속 분석에 있어서 시료의 채취, 보관, 분석에 대한 정확한 인식 및 이해 부족은 자료의 질을 저하시키고 있다. 해양환경 연구 및 환경평가에 활용하기 위하여 자연수 중 미량금속의 정확한 자료를 획득하는 주요 과정에 대하여 소개하고자 한다.

**핵심용어 :** 미량금속, 자연수, 해수, 청결기술, 분석

**Key Words :** The metals such as Al, Ag, Au, Cu, Cd, Co, Fe, Ni, Pb, Zn, etc. are present at very low concentration in seawater and are classified as so-called trace metals. While some of them are used in metabolism of living organism as a micronutrient, they may show toxic effects on organisms in case of a limited threshold concentration of them. Plenty of studies on trace metals have been performed because trace metals have a persistent influence and an adverse effect on marine environment and ecosystem. For long years, when the concentration of trace metals in natural waters such as seawater and fresh water are measured with high precision and accuracy, some systematic errors have been recognized to be present in measurements. Since 1975 in US and European countries, the measured concentration of trace metals in seawater have been found to be lower by factors of 10-1,000 than the previous data of trace metals measurements and the vertical profiles of the measurements have been shown to reflect well-known biological, physical and geochemical processes. These results are attributed to great advances in analytical instrumentation and methodology for trace metals measurements. Precautions against the contamination of samples are required to be taken in the process of sampling, storage, and analysis of samples. However, in Korea, erroneous data of trace metals with regard to ocean and marine environment related survey and investigations are reported. The lack of exact understanding and information regarding precautions in sampling, storage and analysis of samples lead to the deterioration of data quality, especially in the analysis of trace metals. The major procedures to obtain the accurate data of trace metals in natural waters are introduced for applying to the study and assessment of marine environments.

**Key Words :** Trace Metal, Natural Water, Seawater, Clean Technique, Analysis

### 1. 서 론

미량금속(Trace metal)은 일반적으로 해수 및 담수 등의 자연

수 중에 극미량으로 존재하며(Bruland et al., 1991), 미량원소(Trace element), 중금속(Heavy metal)로도 불린다. 미량금속은 원소에 따라 생물에게 필수 원소로 작용하는 경우도 있으나 환경 중에 유입되면 분해되지 않는 특성을 가지고 있으므로 지속성 오염물질로 분류되고 있다. 이들은 생물에 농축(Bioaccumulation)될 뿐 아니라 생태계의 먹이사슬을 통하여

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# Evolution of the Dok Do seamounts, Ulleung Basin, East Sea: constraints based on the reconstruction of virtual geomagnetic poles using paleomagnetic data

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**Abstract** In the Ulleung Basin, East Sea, the Dok Do seamount group comprises Dok Do (Dok Island), consisting of very small islets/rocks and a large submerged volcanic edifice, and two voluminous tablemounts, Simheungtaek and Isabu. We attempted to reconstruct the evolution of these seamounts, using virtual geomagnetic poles (VGPs) determined by the least-squares and the seminorm magnetization methods, with 1,500 m upward continued magnetic anomalies. The VGPs of Dok Do with normal dipole anomaly, and of Simheungtaek with normal dipole anomaly are located near the present magnetic pole. The VGP of Isabu with normal dipole anomaly is located at low latitude, presumably due to overprints of reversals in the Tertiary, and the distortion of magnetization and structures associated with volcanism after its formation. In contrast to the tablemounts, magnetic anomalies over Dok Do are a combination of both normal polarity and reversed polarity dipoles in the northern

hemisphere, indicating that Dok Do has had at least two major eruptions, one during normal and another during reversed polarity intervals. From these results, and information on the ages of the seamounts (either published radiometric ages of subaerial volcanic rocks, or ages reconstructed in terms of reported elastic thickness incorporated into an existing cooling plate model), we tentatively propose that (1) Isabu formed first, during a normal polarity interval after the opening of the East Sea had ceased; (2) this was followed by an initial and subsequent large eruption of Dok Do during a normal polarity and a reversed polarity interval after about 5 Ma; and (3) the formation of Simheungtaek occurred in between that of Isabu and Dok Do in a normal polarity interval. The pattern of normal/reversed magnetization is not inconsistent with the geomagnetic polarity timescale for at least the last 5 Ma. Nevertheless, precise ages of formation would need verification by additional geophysical/geochemical constraints. Evaluating various possible models explaining the successive formation of the Dok Do seamounts, we currently favor fracturing and volcanism related to compression-induced weakening of the extensional field from the late Miocene to Pliocene after the opening of the East Sea.

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## Introduction

The East Sea (Sea of Japan) is a back-arc basin consisting of three deep basins—the Ulleung, Japan, and Yamato basins—surrounded by Korea, Japan, and Russia (Fig. 1) in a complex junction between the Eurasian, Pacific, and Philippine plates (Uyeda and Miyashiro 1974). When the East Sea formed in response to the subduction of the Philippine and Pacific plates, the Japan islands moved away from the Eurasian margin (Hilde and Wageman 1973; Uyeda and Miyashiro 1974). It is generally accepted that

## 독도주변 탄산염퇴적물의 분포와 성인: 예비조사 결과

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## Distribution and Origin of Carbonate Sediments near Dok Island: Preliminary Study

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독도 주변 퇴적물의 구성요소를 분석하여 퇴적물의 기원과 퇴적상을 조사하였다. 퇴적물은 독도를 이루는 화산기의 암편과 천해에서 서식하고 있는 여러 생물체에 의해 생성된 탄산염퇴적물로 구성된다. 대부분 탄산염입자와 화산암편으로 이루어진 퇴적물 중에서 탄산염입자는 연체동물(이매패류와 복족류), 덮개상과 가지상 태선동물, 덮개상과 마디상 홍조류, 유공충, 해면동물, 환형동물, 극피류(성게류), 절지동물(따개비류), 규조류 등에 의해 만들어지고 있다. 퇴적물 내 구성요소의 상대적 함량의 차이는 주로 수심에 의해 구분되지만 비슷한 수심구간 내에서도 지역적으로 차이를 보이기도 한다. 특히 독도 주변의 퇴적물은 전형적인 냉수성 탄산염퇴적물(cool water carbonates)의 성분을 보여주며, 수심별로 뚜렷한 퇴적상의 변화를 보인다. 독도 주변 퇴적물은 수심에 따른 구성요소 함량의 차이와 입자의 크기에 의해 크게 5개의 퇴적상으로 구분된다. 이들은 해안퇴적상(20 m 이내의 천해퇴적물), 천해퇴적상(20~100 m 구간의 천해퇴적물), 상부 전이퇴적상(100~200 m의 퇴적물), 하부 전이퇴적상(200~700 m의 퇴적물), 그리고 반원양퇴적상(700 m 이상의 반원양성 퇴적물)이다. 수심 2,000 m 이상의 퇴적물 내에도 약 10~20%의 탄산염퇴적물이 함유되어 있으며, 이는 독도 주변 해역의 탄산염보상심도가 이보다 더 깊을 수 있음을 암시한다.

Based on the constituent analysis of sediments near Dok Island, the origin and sedimentary facies were investigated. The sediments are mainly from originated from volcanic and volcanoclastic rock fragments derived from Dok Island and carbonate sediments formed by a variety of shallow-dwelling organisms that secreted calcareous skeletons. Carbonate producers include mollusks (bivalves and gastropods), encrusting & branching bryozoans, encrusting & segmented red algae, worm tubes, barnacles, diatoms, sponge spicules and echinoderm fragments. The distribution and relative amount of these constituents are basically dependent upon water depth and grain size even though local variations can be observed within the same depth interval. Five sedimentary facies can be divided: nearshore facies (<20 m), neritic facies (20~100 m), upper transitional facies (100~200 m), lower transitional facies (200~700 m), and hemipelagic facies (>700 m). The sediments that were sampled below the water depth of 2,000 m still contain a significant amount of carbonates (ca. 10~20%), implying that the carbonate compensation depth in the East Sea may well exceed this water depth.

**Keywords:** Cool Water Carbonates, Dok Island, Sedimentary Facies, East Sea, Carbonate Compensation Depth

### 서 론

탄산염퇴적물은 그 기원과 퇴적작용이 쇄설성 퇴적물과는 많은 차이가 있다. 특히 천해지역에서 퇴적되는 탄산염퇴적물과 쇄설성 퇴적물의 가장 큰 차이는 탄산염퇴적물의 기원이 대부분 생물기원이며, 쇄설성퇴적물과는 달리 퇴적물이 생성된 분지 내에서 주로

퇴적된다는 것이다(유와 우, 2007). 즉 탄산염퇴적물이 퇴적되는 지역은 쇄설성퇴적물에 비해 지역적으로 매우 제한적이다. 탄산염퇴적물이 퇴적되기 위해서는 우선 탄산염 골격을 생산하는 생물(주로 무척추동물이나 식물)이 천해에 퇴적물로서 쌓일 정도로 충분히 살고 있어야 하며, 쇄설성 입자의 유입이 제한적으로만 이루어지는 지역이어야 한다(Pedley and Grasso, 2002). 1960년대에서 1970년대에 해양학자들은 현생 천해환경을 조사하면서 탄산염퇴적물이 주로 열대와 아열대의 수온이 높고 물의 탁도가 낮은 환경에

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## RESEARCH ARTICLE

# Phylogenetic diversity of *Synechococcus* strains isolated from the East China Sea and the East Sea

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## Keywords

*Synechococcus*; East Sea; East China Sea; phylogeny; phycoerythrin.

## Abstract

Phylogenetic relationships among 33 *Synechococcus* strains isolated from the East China Sea (ECS) and the East Sea (ES) were studied based on 16S rRNA gene sequences and 16S–23S rRNA gene internal transcribed spacer (ITS) sequences. Pigment patterns of the culture strains were also examined. Based on 16S rRNA gene and ITS sequence phylogenies, the *Synechococcus* isolates were clustered into 10 clades, among which eight were previously identified and two were novel. Half of the culture strains belonged to clade V or VI. All strains that clustered into novel clades exhibited both phycoerythrobilin and phycourobilin. Interestingly, the pigment compositions of isolates belonging to clades V and VI differed from those reported for other oceanic regions. None of the isolates in clade V showed phycourobilin, whereas strains in clade VI exhibited both phycourobilin and phycoerythrobilin, which is in contrast to previous studies. The presence of novel lineages and the different pigment patterns in the ECS and the ES suggests the possibility that some *Synechococcus* lineages are distributed only in geographically restricted areas and have evolved in these regions. Therefore, further elucidation of the physiological, ecological, and genetic characteristics of the diverse *Synechococcus* strains is required to understand their spatial and geographical distribution.

## Introduction

*Synechococcus* are ubiquitously distributed throughout the world's oceans, ranging from equatorial to polar waters as well as from coastal to open waters. This wide distribution of *Synechococcus*, as opposed to *Prochlorococcus*, which live in oligotrophic open waters (Partensky *et al.*, 1999), might be due to their ability to populate marine surface waters over a wide range of environmental conditions (Penno *et al.*, 2006). Indeed, phylogenetic and physiological data have shown that *Synechococcus* comprise a very diverse group (Rocap *et al.*, 2002; Fuller *et al.*, 2003; Ahlgren & Rocap, 2006).

Phylogenetic analyses of *Synechococcus* have been performed using genetic markers including 16S rRNA gene, the 16S–23S rRNA gene internal transcribed spacer (ITS), the RNA polymerase gene (*rpoC1*), the phycoerythrin gene (*cpeB*), the nitrate reductase gene (*narB*), and the nitrogen regulator gene (*ntcA*) using cultured and natural samples from marine environments (Toledo & Palenik, 1997; Rocap *et al.*, 2002; Fuller *et al.*, 2003; Mühling *et al.*, 2005; Ahlgren

& Rocap, 2006; Jenkins *et al.*, 2006; Penno *et al.*, 2006; Haverkamp *et al.*, 2008; Paerl *et al.*, 2008). To date, 12 distinct *Synechococcus* lineages from culture isolates and at least four additional lineages from environmental clones have been described (Rocap *et al.*, 2002; Fuller *et al.*, 2003; Ahlgren & Rocap, 2006; Penno *et al.*, 2006). Some of these phylogenetic clades can be differentiated from others by physiological traits such as motility (clade III), lack of accessory pigments, phycourobilin (clade VI) or both phycoerythrobilin and phycourobilin (clade VIII), ability of chromatic adaptation (clades I, III, IV, IX, XV, and XVI), and reduced efficiency in nitrate utilization (clades CRD1 and XV) (Toledo *et al.*, 1999; Palenik, 2001; Moore *et al.*, 2002; Fuller *et al.*, 2003; Ahlgren & Rocap, 2006; Six *et al.*, 2007).

It is well accepted that the distribution of *Synechococcus* lineages varies spatially and temporally (Fuller *et al.*, 2003, 2005; Penno *et al.*, 2006). Given that clades are hypothesized to represent physiologically and ecologically distinct populations or ecotypes (Ahlgren & Rocap, 2006; Dufresne *et al.*, 2008), the distribution of *Synechococcus* likely varies in time and space to enable survival in each environmental



## Flexural isostasy and loading sequence of the Dokdo seamounts on the Ulleung Basin in the East Sea (Sea of Japan)

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### ABSTRACT

The isostatic compensation and loading sequence of the Dokdo seamounts on the Ulleung Basin in the East Sea are studied by a flexure model using gravity and bathymetric data. The Dokdo seamounts comprise Dokdo (Dok Island), lying as a cluster of emerged small islets surmounting a large submerged volcanic edifice, and two voluminous tablemounts, Simheungtaek and Isabu. In spite of their similarity in topographic expression, a large difference of about 50 mGal between the observed gravity anomaly peaks over Dokdo and the Isabu Tablemount implies different amounts of compensation. A comparison between the observed and calculated gravity anomalies of the seamounts shows that the flexural compensation investigated in this study predicts well the observed data. The flexure model suggests that the Dokdo seamounts might be composed of high-density volcanic rocks. Modeling results show that the flexural rigidity or effective elastic thickness of the lithosphere under Dokdo is stronger or thicker than that under the Isabu Tablemount. It implies that the age of lithosphere at the time of loading for Isabu was younger than that for Dokdo. The effective elastic thicknesses associated with the Dokdo seamounts approximately follow 200–400 °C isotherms in the cooling plate model. The results of this study and the former age dating of Dokdo suggest that the Isabu Tablemount was formed first, followed by Dokdo after the opening of the East Sea ceased.

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### 1. Introduction

The Ulleung, Japan, and Yamato deep basins comprise a back-arc basin beneath the East Sea (Sea of Japan). Korea, Japan, and Russia share the surrounding shores. Although the origin of the East Sea has been debated by many scientists, it is generally accepted that its opening was initiated in the early Miocene and then ceased about 15–12 Ma (Lee et al., 1999; Itoh, 2001).

Among the three basins, the Ulleung was formed during the last stage of the opening of the East Sea (Lallemand and Jolivet, 1986; Tatsumi et al., 1989). The Ulleung Basin is separated on the north from the Japan Basin by the Korean Plateau and northeastward the Oki Bank distinguishes it from the Yamato Basin. The crust under the Ulleung and Yamato basins is disputably oceanic or continental (Jolivet et al., 1991; Kim et al., 1994; Park, 1998). The results and interpretation of ocean bottom seismometry, gravity, magnetic, and other seismic observation data suggest that the Ulleung Basin may be characterized as having a thick oceanic crust possibly representing a spreading center (Kim et al., 1994; Park, 1998; Lee

et al., 1999). The Ulleung Basin is known to have very poor magnetic lineation, which may be attributed to an anomalously thick crust or high heat flow (Isezaki and Uyeda, 1973; Kurashimo et al., 1996). Scientists have suggested several hypotheses for the origin of the abnormally thick oceanic crust (Hirata et al., 1989; Jolivet and Tamaki, 1992; Tamaki et al., 1992; Lee et al., 1999).

Our study area is the northeastern part of the Ulleung Basin where Dokdo (Dok Island), lying as a cluster of emerged small islets surmounting a large submerged volcanic edifice, occurs together with the adjacent Simheungtaek and Isabu tablemounts (Song et al., 2000, see Figs. 1 and 2). Dokdo is composed of volcanic rocks such as alkali basalt, trachyte, and trachyandesite (Won and Lee, 1984; Sohn and Park, 1994). Rock samples from the subaerial portion of Dokdo were dated at  $4.6 \pm 0.4$  Ma in the early Pliocene to  $2.7 \pm 0.1$  Ma in the late Pliocene to Quaternary (Sohn and Park, 1994). The submerged parts of the island and the seamounts are less studied because of their difficult access.

Gravity and bathymetric data are widely used for a better understanding of the tectonic processes associated with underwater igneous edifices such as the Hawaiian seamounts and the Ontong Java Plateau (Ito and Taira, 2000; Watts, 2001). A flexure model is frequently applied to allow the interpretation of compen-

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## *Salinisphaera dokdonensis* sp. nov., isolated from surface seawater

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A Gram-negative, strictly aerobic bacterium, designated CL-ES53<sup>T</sup>, was isolated from surface water of the East Sea in Korea. Cells of strain CL-ES53<sup>T</sup> were short rods and motile by means of monopolar flagella. Strain CL-ES53<sup>T</sup> grew with 4–21 % NaCl (optimum 10 %) and at 5–40 °C (optimum 25 °C) and pH 5.2–8.8 (optimum pH 6.3–7.2). The major isoprenoid quinone was Q-8. The major fatty acids were C<sub>18:1</sub>ω7c (42.0 %), C<sub>18:1</sub>ω9c (14.8 %) and C<sub>14:0</sub> (9.4 %). The genomic DNA G + C content was 64.9 mol%. Analysis of the 16S rRNA gene sequence of strain CL-ES53<sup>T</sup> revealed that it was a member of the genus *Salinisphaera* and most closely related to *Salinisphaera shabanensis* E1L3A<sup>T</sup> (96.9 % sequence similarity) and *Salinisphaera hydrothermalis* EPR70<sup>T</sup> (93.8 %). Phylogenetic analyses based on the 16S rRNA gene sequence showed that strain CL-ES53<sup>T</sup> formed a robust cluster with *S. shabanensis* E1L3A<sup>T</sup>. Although the 16S rRNA gene sequence similarity between strain CL-ES53<sup>T</sup> and *S. shabanensis* E1L3A<sup>T</sup> was rather high (96.9 %), DNA–DNA relatedness between these strains was 12 %, suggesting that they represent genomically distinct species. Strain CL-ES53<sup>T</sup> was differentiated from *S. shabanensis* E1L3A<sup>T</sup> and *S. hydrothermalis* EPR70<sup>T</sup> on the basis of optimum temperature for growth and certain phenotypic characteristics. The phylogenetic analysis and physiological and chemotaxonomic data show that strain CL-ES53<sup>T</sup> should be classified in the genus *Salinisphaera* within a novel species, for which the name *Salinisphaera dokdonensis* sp. nov. is proposed. The type strain is CL-ES53<sup>T</sup> (=KCCM 90064<sup>T</sup> =DSM 19549<sup>T</sup>).

The genus *Salinisphaera* in the class *Gammaproteobacteria* was established by Antunes *et al.* (2003) with *Salinisphaera shabanensis* as the type species. Until now, two *Salinisphaera* strains have been isolated from extreme environments: *S. shabanensis* E1L3A<sup>T</sup> was isolated from the brine–seawater interface (1331 m depth, 21–23 % salinity) of the Shaban Deep in the Red Sea (Antunes *et al.*, 2003) and *Salinisphaera hydrothermalis* EPR70<sup>T</sup> was isolated from the deep-sea hydrothermal vents (about 2500 m depth) on the East Pacific Rise (Crespo-Medina *et al.*, 2009). Cells of *S. shabanensis* E1L3A<sup>T</sup> are monotrichous cocci that show a remarkable physiological flexibility, as seen by quite broad growth ranges for oxygen (anaerobic and aerobic), temperature (5–42 °C, optimum 30–37 °C) and NaCl (1–28 %, optimum 10 %) (Antunes *et al.*, 2003). Cells of *S. hydrothermalis* EPR70<sup>T</sup> are short rods that are motile by means of one or more flagella, strictly aerobic, mesophilic (30–35 °C, optimum 20–40 °C), halotolerant (1–25 % NaCl, optimum 2.5 % NaCl) and facultatively chemolithoautotrophic (Crespo-Medina *et al.*, 2009). This study describes a strictly aerobic, moderately halophilic,

short-rod-shaped bacterium, strain CL-ES53<sup>T</sup>, which was isolated from the surface of offshore seawater.

Seawater was collected from the surface in the vicinity of Dokdo, an island in the East Sea, Korea. An aliquot (100 µl) of seawater was spread on MY solid medium, which was used to isolate polysaccharide-producing bacteria by Bouchotroch *et al.* (2001), and incubated under aerobic conditions at 30 °C for 1 week. Strain CL-ES53<sup>T</sup> was isolated and subsequently purified on marine agar (MA; Difco) at 30 °C, because strain CL-ES53<sup>T</sup> grew well on both MA and MY plates, by picking a single colony and streaking it onto fresh MA four times. The strain was maintained on MA at 30 °C and preserved in marine broth 2216 (MB; Difco) supplemented with 30 % (v/v) glycerol at –80 °C.

For 16S rRNA gene sequence amplification by PCR, DNA was extracted from a single colony by the boiling method (Englen & Kelley, 2000). The crude extracts served as the DNA template for PCR, which used *Taq* DNA polymerase (Promega) and primers 27F and 1492R (Lane, 1991). PCR amplification products were purified by using the AccuPrep PCR purification kit (Bioneer) and direct sequence determination of the purified amplification products was performed using sequencing primers (27F,

Abbreviation: PHB, poly-β-hydroxybutyrate.

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain CL-ES53<sup>T</sup> is EF988634.