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研究課題名（和文）Microbial Communities in the Sea Surface Microlayer as a Potential Source of Biogenic Ice Nuclei in Oceanic Regions

研究課題名（英文）Microbial Communities in the Sea Surface Microlayer as a Potential Source of Biogenic Ice Nuclei in Oceanic Regions

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研究成果の概要（和文）：海洋の極表層にはマイクロレイヤー（SML）と呼ばれる微生物が濃縮した層が存在する。SMLは海洋 大気間の物質循環、気候や放射収支に対する海洋エアロゾルの役割などを知る上で重要だと考えられるが、その微生物学的特徴について未だ知見に乏しい。本研究では沿岸域SML中の氷核形成細菌の動態を解析し、SMLが気候や大気プロセスに与える影響について明らかにした。

研究成果の学術的意義や社会的意義

Ice nucleation particles play an important role in regulating oceanic and atmospheric processes such as cloud formation. Ice nucleation bacteria isolated and characterized from this study can help with the understanding on how these bacteria contributes or affect the atmospheric processes.

研究成果の概要（英文）：The lack of understanding and research in the field of sea surface microlayer (SML) hampers the effort to predict the contribution of marine aerosol towards the global climate and radiation budget accurately. This statement rings true as well for the field of ice nucleation study especially in marine environment, whereby previous research has shown that these particles have the ability to control cloud lifetime, precipitation and radiative properties. In short, this study on the microbial dynamics in coastal SML with regards to ice-nucleating bacteria will provide significant insights into the yet-to-be-studied microbial roles in controlling atmospheric processes over large swath of ocean as well as regional climate.

研究分野：微生物分野

キーワード：Sea Surface Microlayer Ice Nucleation Particles Ice Nucleation Assay

1. 研究開始当初の背景

The lack of understanding and research in the field of the sea surface microlayer (SML) hampers the effort to predict the contribution of marine aerosol towards the global climate and radiation budget accurately. This statement rings true as well for the field of ice nucleation study especially in marine environment, whereby previous research has shown that these particles have the ability to control cloud lifetime, precipitation and radiative properties. In short, this study on the microbial dynamics in coastal SML with regards to ice-nucleating bacteria will provide significant insights into the yet-to-be-studied microbial roles in controlling atmospheric processes over large swath of ocean as well as regional climate.

2. 研究の目的

The purpose of this research project is to understand microbial dynamics in the SML and sea spray aerosols (SSA), especially in the aspect of microbial export to the atmosphere and its effect on the ice nucleation particle (INP) formation. *All of these are still novel to the field of atmospheric science as well as the field of SML studies, especially when related to the microbiology of these habitats.* This proposed research would add more new perspective into this field with the following originality and scientific significance: *ab) Elucidating the INP potential in the SML and UW.* INPs originating from SSA are able to control the radiative properties and cloud cover throughout a large regional scale. Moreover, the biological IN activity are usually enhanced under nutrient limited conditions and hence the importance of quantifying the INP in the seawater; *b) Narrowing down the microbial community in the SML and UW actually responsible for the IN activity in each respective layer.* The actual microbial community in the SML and UW responsible to produce INP are still unknown. But the SML being at the top most layer of the water column and concentrated with a variety of materials including microorganisms would definitely be one of the main contributors to IN activity in the ocean.

3. 研究の方法

Samplings were carried out at the pier of the Misaki Marine Biological Station. Water samples were collected from the SML using polycarbonate membrane, glass plate and drum sampler. Underlying water were collected at the depth of 20cm for comparison. Bacteria isolated from the water samples were inoculated onto 1/2-strength Zobell agar medium. DNA were extracted from the pure isolates and were sequenced to identify the strains. The identified bacterial strains were then subjected to ice nucleation assay (INA) test by freezing the bacterial droplets at -1°C to -30°C with an interval of 1°C, with a holding time of 10mins at each temperature. INA positive bacteria were then subjected to heat, proteinase K treatment and filtration tests to characterize the ice nucleation protein present. The amount of ice nucleation particles for each sample was also quantified.

4. 研究成果

A total of 92 strains (Table S1) were tested for their ability to freeze PBS using droplet-freezing assay. Out of the 92 strains tested, only four strains that had at least four out of eight droplets

frozen at -10°C (Fig. 1) were considered as potential ice nucleus and were chosen for further. Several treatments were performed to test the property and nature of INA positive bacteria. All six strains tested in this study were sensitive to heat and their ice nucleation activity diminished upon heating when compared to unheated samples. All the tested samples had lower INA activity upon heat treatment as heat appears to inactivate the INA activity for INA positive samples to some extent.

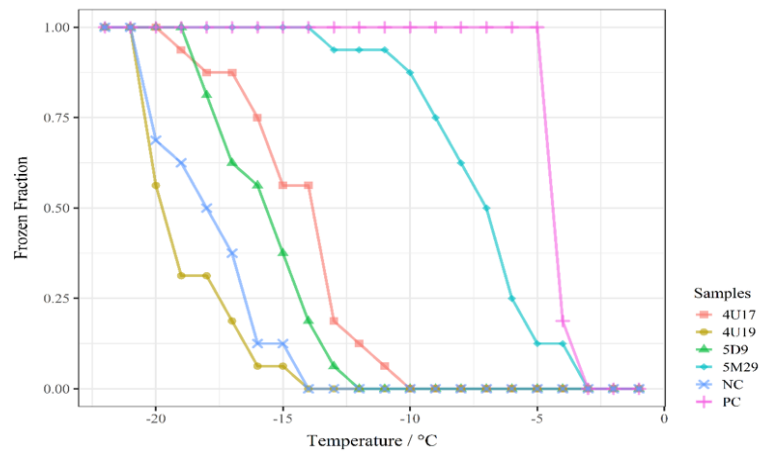


Figure 1. Frozen fractions of four potential ice nucleation bacteria out of 92 tested strains using the semi-quantitative droplet-freezing assay.

To determine the INA potential after size fragmentation, cell suspensions were filtered through a 100kDa membrane. The INA Positive strains 5M29 and 5G21 (Fig.2) showed significant difference in freezing temperature in the solutions retained above the membrane when compared to the control samples. On the other hand, the filtrate for these three strains showed no significant differences in freezing temperatures. This indicates that the solution on the top of membrane contained molecules which induced the ice nucleation and its size is presumably >100 kDa.

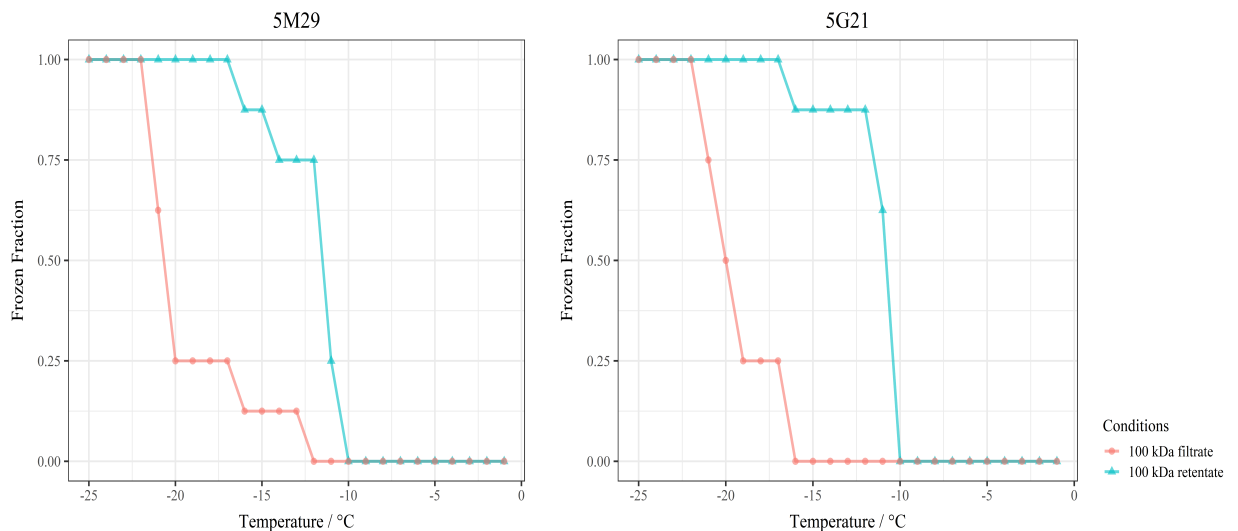


Figure 2. The frozen fraction of strains 5M29 and 5M3 in the retentate and filtrate fractions.

Proteinase K was used to cleave the peptide bonds in INA protein. In this treatment, five out of six strains tested (4U17, 4U19, 5M3, 5G21 and 5M29) showed significant decrease in the freezing temperature. Among all the INA positive strains, 5M29 showed the highest ice nucleation activity. The amount of ice nucleation particles in strain 5M29 were further quantified (Fig.3).

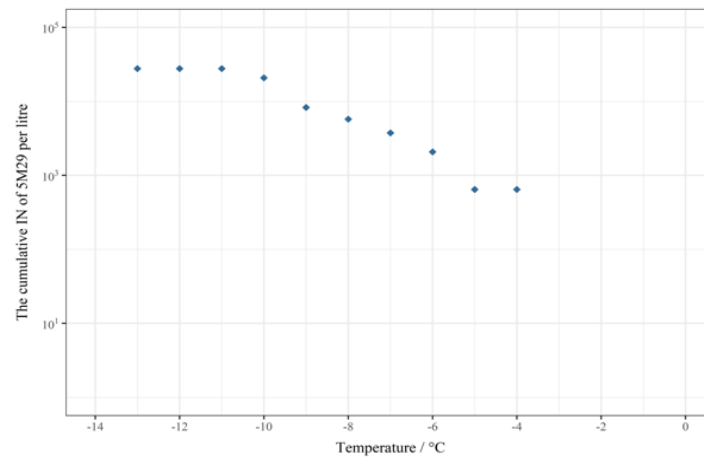


Figure 3. The cumulative IN of 5M29 from -4 °C to -13 °C tested using droplet-freezing assay.

The result in this study verifies that the ice nucleation induced by most of INA bacteria is biogenic origin and is induced by INA protein and agrees with the study of Pouleur et al. (1992). However, further studies should aim to identify the structure of INA molecule found in this study. The finding in this study showed that, similar to other environments, sea spray aerosols generated by bubble bursting of seawater and the SML can also contain ice nucleating bacteria which can act as ice nuclei and catalyze ice formation in the atmosphere.

5. 主な発表論文等

〔雑誌論文〕 計3件（うち査読付論文 3件/うち国際共著 3件/うちオープンアクセス 3件）

1. 著者名 Wong Shu-Kuan, Suzuki Shotaro, Cui Yingshun, Kaneko Ryo, Kogure Kazuhiro, Hamasaki Koji	4. 巻 8
2. 論文標題 Sampling Constraints and Variability in the Analysis of Bacterial Community Structures in the Sea Surface Microlayer	5. 発行年 2021年
3. 雑誌名 Frontiers in Marine Science	6. 最初と最後の頁 696389
掲載論文のDOI（デジタルオブジェクト識別子） なし	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

1. 著者名 Fernandes Sheryl Oliveira, Gonsalves Maria Judith, Nazareth Delcy Rosy, Wong Shu-Kuan, Haider Md Nurul, Ijichi Minoru, Kogure Kazuhiro	4. 巻 270
2. 論文標題 Seasonal variability in environmental parameters influence bacterial communities in mangrove sediments along an estuarine gradient	5. 発行年 2022年
3. 雑誌名 Estuarine, Coastal and Shelf Science	6. 最初と最後の頁 107791 ~ 107791
掲載論文のDOI（デジタルオブジェクト識別子） なし	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

1. 著者名 Cui Yingshun, Wong Shu-Kuan, Kaneko Ryo, Mouri Ayako, Tada Yuya, Nagao Ippei, Chun Seong-Jun, Lee Hyung-Gwan, Ahn Chi-Yong, Oh Hee-Mock, Sato-Takabe Yuki, Suzuki Koji, Fukuda Hideki, Nagata Toshi, Kogure Kazuhiro, Hamasaki Koji	4. 巻 11
2. 論文標題 Distribution of Dimethylsulfoniopropionate Degradation Genes Reflects Strong Water Current Dependencies in the Sanriku Coastal Region in Japan: From Mesocosm to Field Study	5. 発行年 2020年
3. 雑誌名 Frontiers in Microbiology	6. 最初と最後の頁 1372
掲載論文のDOI（デジタルオブジェクト識別子） 10.3389/fmicb.2020.01372	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕 計5件（うち招待講演 1件/うち国際学会 4件）

1. 発表者名 山口量平
2. 発表標題 波の花に由来する氷核活性細菌の探索
3. 学会等名 海洋生物学研究会シンポジウム
4. 発表年 2019年

1. 発表者名 濱崎恒二
2. 発表標題 Isolation and Characterization of Ice-Nucleating Bacteria in Seawater and Seafoam Collected from the Coast of Noto Peninsula, Japan
3. 学会等名 Asian Symposium on Microbial Ecology (国際学会)
4. 発表年 2019年

1. 発表者名 Koji Hamasaki
2. 発表標題 Dynamics of microbes and organic matter at the air-sea interface: insights from an observational study in Tsukumo Bay, Noto Peninsula, Japan.
3. 学会等名 INET Joint Usage Research Symposium (招待講演) (国際学会)
4. 発表年 2019年

1. 発表者名 土橋 司
2. 発表標題 北太平洋亜熱帯域における海洋大気エアロゾル中の水溶性有機態窒素の起源.
3. 学会等名 日本地球惑星科学連合 (国際学会)
4. 発表年 2019年

1. 発表者名 Hamasaki Koji
2. 発表標題 Microbial community dynamics in sea surface microlayer and sea spray aerosols observed in coastal inlets of Japan.
3. 学会等名 SOLAS Open Science (国際学会)
4. 発表年 2019年

〔図書〕 計0件

〔産業財産権〕

〔その他〕

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6. 研究組織

	氏名 (ローマ字氏名) (研究者番号)	所属研究機関・部局・職 (機関番号)	備考
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7. 科研費を使用して開催した国際研究集会

〔国際研究集会〕 計0件

8. 本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関
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