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Abstracts

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OBo1

Isolation and characterization of Antarctic actinobacteria with antimicrobial activities

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The ability of Antarctic microorganisms to produce antimicrobial agents has conferred a competitive edge to these microorganisms in the harsh and nutrient-deprived environment. Actinomycetes strains are the major producer of antimicrobial compounds, such as antibiotics. However, the isolation and analysis of antimicrobial compounds from Antarctic actinomycetes are relatively low when compared to those found in other parts of the world. Hence, the objectives of this work were to isolate and characterize the antimicrobial compounds of Antarctic actinomycetes from Ardley Island, Antarctica. We have isolated two new actinomycetes strains, INACH3013a and INACH3013b, which displayed antimicrobial properties from soil samples collected from Ardley Island. They were identified by sequencing and analysis of their 16S rDNA and were found to be closest to the Streptomyces spp. The results showed that strain INACH3013 and INACH3013a were closest to Streptomyces fildesensis, and Streptomyces spp., respectively. Strain INACH3013b was closest to Streptomyces corallincola and Streptomyces bullii. The extracellular compounds they produced were extracted using various solvents and the extracted compounds were tested against the test bacterial pathogens. After preliminary determination, strain INACH3013, strain INACH3013A and INACH3013b all demonstrated antibacterial properties, with INACH3013A showing the most potent effect. Their intracellular and extracellular compounds were extracted using dichloromethane (DCM) and tested against 18 Gram-positive or Gram-negative test pathogens. Subsequently, bioactive substances Minimum Inhibitory Concentration (MIC) were determined, and the bioactive compounds were separated using Thin Layer Chromatography (TLC). The findings indicated that there were a few compounds from the TLC of strain INACH3013a that produced very potent antibacterial compounds and could be novel antibiotic compounds. Those compounds will be purified and structurally characterized in the future to determine their novelties.

Reference

Lavin, P. L., Yong, S. T., Wong, C. M., & De Stefano, M., Isolation and characterization of Antarctic psychrotroph *Streptomyces* sp. strain INACH3013, Antarctic Science, 28(6), 433-442, 2016

Comprehension of Plankton diversity and biogeochemical processes in the Polar ecosystem climate change scenario

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The global environmental changes due to natural and anthropogenic impacts are challenging the structure and functioning of the ocean food web ecosystem. The composite processes interacting within the physical, chemical, and biological environment at different spatio-temporal scales and their influence on the ocean ecosystem processes are yet to be explored. A long-term trend on phytoplankton biomass (in terms of Chlorophyll-a concentrations; Chl-a), phytoplankton compositions and the processes that regulate the variability is required for understanding the ocean ecosystem. The present research investigated decadal trends of phytoplankton compositions and biogeochemical variables over the Global Ocean (GO), Southern Ocean (SO), and the Arctic Ocean (AO) using ocean color remote sensing and assimilated data from the National Aeronautics and Space Administration (NASA) Ocean biogeochemical model. The consequences revealed the dominance of diatoms (larger cell) throughout the SO and AO; however, the coccolithophores dominate in the remaining part of the GO. Analysis of nutrients indicated that nitrate is not a limiting for the variability of phytoplankton biomass in the SO and AO. The low nitrate concentration influenced in the rest of the GO. The photosynthetically available radiation (PAR) is limiting the phytoplankton biomass and compositions in the SO and AO. Although the SO is known as the high nutrient low chlorophyll (HNLC) region of the GO, the low iron concentration along with the PAR colimits the growth of phytoplankton biomass. Trend analysis displayed an increase in Chl-a and diatoms in the SO and AO. In contrast, it declined significantly in the other regions of the GO, in response to the consistent increase in sea surface temperature. The results indicated that, shifting of phytoplankton community from regional to global scale have a greater implication for climate change and marine food web ecosystem.



Fig.1: Phytoplankton biomass and various communities (a) Distribution and their trends in Global Ocean

(b) distribution and their trend in the Southern Ocean during 2002-2015.

The phytoplankton biomass i.e Chlorophyll-*a* (Chl-*a*) varied a modest in GO and SO but higher in AO. The Chl-*a* varied from 0.28 mg m⁻³ to 0.31 mg m⁻³ (mean ± standard error, SE) of 0.29 ± 0.002 mg m⁻³ in GO (Fig.1&2), from 0.27 mg m⁻³ to 0.32 mg m⁻³ of (mean ± SE; 0.028 ± 0.005 mg m⁻³) in SO and from 1.03 mg m⁻³ to 1.18 mg m⁻³ of (mean ± SE; 1.09 ± 0.013 mg m⁻³) in AO (Fig.2) respectively. The Chl-*a* trend significantly increased in AO (p = 0.0161) during 2002–2015. However, the trend was a negligible increase with no significance in SO (p = 0.4033) and the trend was negative with no significance in GO (p = 0.2496). As observed in the physical-chemical parameters, the Chl-*a* concentration and various phytoplankton community structure was varied due to various water mass occurrences between GO, AO and SO in the sea ice free zones.

Fig.2: Phytoplankton biomass and various communities distribution and their trend in the Arctic Ocean during 2002-2015.

Statistically the decline of Chl-*a* and diatoms are significant during study period in GO. While the Chl-*a* and diatoms trend increased in SO and the AO, the increment is not statistically significant (Fig.1) in SO. The results indicate approximately 75% of Chl-*a* (Fig.1) in GO has a decreased trend. In addition to this, it is also a fact that the Chl-*a* of GO and other oceans has a declined trend, was spatially diverse and varied greatly in respect to the seasons. However, in the present

study the Chl-*a* and diatoms distribution in AO are highly heterogeneous and the trends are statistically significant (Fig.2). The AO is being warmed where rising the surface atmospheric heat fluxes and accelerating the ice cover led to increase the ice melting and freshening is occurring during the summer (Zhang, 2005). Results, the strong stratification which prevents the upcoming of nutrient and thus limits the Chl-*a* and the productivity. However, the present investigation made for the annual average status of Chl-*a* and diatoms trend, that increased probability with the consistency of MLD and hence the renewal of the winter nutrient occurred through strong mixing which propagate the growth of Chl-*a* and the diatoms. The increase of Chl-*a* and diatoms simultaneously exhibits the maximum and diatoms contribution is higher in AO than other communities due to the wide adaptation to temperature and PAR in high latitude (Mishra et al., 2015). Although the PAR and nitrate are almost consistent and the trends show barely any variation, the increase trend of micronutrient iron plays a major role in the allocation of Chl-*a* and diatoms. The reason for annual average distribution of Chl-*a* and diatoms along with significant increases of their trends in AO during the study period are ruled out to compare the annual average trend of SO with similar environmental conditions.

The cumulative effect of the physical, chemical and biological mechanism that drive the variations of the community are needed to be understood appropriately, however the present study result would make available for first line information and are an indicator of the variability that appeared in trends. Declining of global Chl-*a*, increasing trend of cyanobacteria and increasing trend of SST consistent lead to the hypothesis that the ocean warming is causing to reform a marine ecosystem with an implication of biogeochemical cycling, primary productivity, fishery productivity and ocean circulation. To understand the consequences of biogeochemical implications there is a need of generous further in-situ data of nutrients and phytoplankton community to validate the biogeochemical model in the regional and global scale to reduce the uncertainties for future predictions.

References

1.W.W. Gregg, C.S. Rousseaux (2014).Decadal trends in global Pelagic ocean chlorophyll: a new assessment integrating multiple satellites, in situ data, and models. J. Geophys. Res. Oceans., 119 pp. 5921-5933

2.V.J. Hill, B. Light, M. Steele, R.C. Zimmerman (2018).Light availability and phytoplankton growth beneath arctic sea ice: integrating observations and modelingJ. Geophys. Res. Oceans., 123 (5) pp. 3651-3667

3.S.L. Hinder, G.C. Hays, M. Edwards, E.C. Roberts, A.W. Walne, M.B. Gravenor (2012). Changes in marine dinoflagellate and diatom abundance under climate changeNat. Clim. Change, 2 (4) pp. 271-275.

4. Zhang and T.J. Zhang (2005). Influence of the seasonal snow cover on the ground thermal regime: an overview. Rev. Geophys.

5. Mishra. R.K., Naik, R.K & N. Anilkunar (2015). Adaptations of phytoplankton in the Indian ocean sector of the Southern Ocean during Austral summer of 1998—2014. Front. Earth Sci., 9 (4), pp. 742-752

Species composition and assemblage characteristics of fish in the shallow coastal waters of the South Bay, Livingston Island (South Shetland Islands, Antarctica)

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The study presents data about the diversity and assemblage structure of fish species in the relatively poorly studied shallow coastal waters of Livingston Island. A total of 624 Notothenioidei fish belonging to seven species were found and measured in the South Bay near the Bulgarian Antarctic base. The material was caught at a depth of up to 25 m, using Nordic-type bottom gill nets in January and February of 2020, 2023, and 2024. The most numerous species in the study area were *Notothenia rossii* (n=422) and *Notothenia coriiceps* (n=147) followed by *Trematomus newnesi* (n=23). The largest number of specimens (n=254) were caught at a depth of 10-15 m belonging to six different species. The dominant species found in this horizon are *N. rossii* (n=175) and *N. coriiceps* (n=56). A total of 219 specimens were caught in the shallowest waters at a depth of 0-10 m, where only 2 species were found – *N. rossii* (n=152) and *N. coriiceps* (n=67). The greatest species diversity was found in the deep areas at a depth of 15-25 m – 146 specimens belonging to 7 species. Dominant species like *T. newnesi*, *T. bernacchii*, and *Lepidonotothen nudifrons* occurred only in the bigger depths where they avoided the predation and concurrence of the two dominant Notothenia species whose numbers are decreasing with the depth. The icefish *Chaenocephalus aceratus* and the dragonfish *Parachaenichthys charcoti* are also found only in the bigger depths, mainly connected to their biology and spawning habitats. Data about length and weight, as well as frequency of occurrence, relative abundance, and relative biomass of all caught fish species are also calculated and presented in the study.

Recent investigations of ichthyofauna in the Barents and Kara Seas

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The Barents and Kara Seas, located in the Arctic region of Russia, have been subjects of ichthyofaunal investigations since the early 20th century. Research in the Barents Sea began in the 1920s, while investigations in the Kara Sea started in the 1930s. However, after the monographs by Andriashev (1954) and Esipov (1952) summarized these results, these investigations became fragmentary and irregular for a long period. The primary focus during this time was on commercially important species. A new annotated list of fish species in the Arctic, including the Barents and Kara Seas, was published only in 1995 by Andriashev and Chernova (1995). This list compiled information from scientific publications up to that period. Since the mid-1990s, the Polar Research Institute of Marine Fisheries and Oceanography (PINRO) (since 2019 - the Polar Branch of the Russian Federal Institute of Fisheries and Oceanography (VNIRO)) has been actively collecting data on all fish species in the Barents Sea (since 2000s with cooperation with the Institute of Marine Research in Norway). Similar surveys were initiated in the Kara Sea starting in 2006. These surveys have provided a comprehensive dataset on the species composition and distribution of ichthyofauna in these seas. In addition, some other Russian academic institutions such as the Murmansk Marine Biological Institute (MMBI) and the Zoological Institute have also conducted ichthyological research in these regions.

The Barents Sea

Since mid-1990s PINRO have conducted annually 3-7 scientific surveys covering almost entire or large part of the Barents Sea. After this preliminary recent general results on species composition of the Barents Sea ichthyofauna based on new actual data were published in 2000s. Totally 207 fish species from 66 families were registered for the Barents Sea as the large marine ecosystem (LME) (Dolgov, 2004), while 182 species from 59 families – for the Barents Sea in its geographical borders (Karamushko, 2008).

Increasing of number of species in the Barents Sea was caused by three main reasons.

Firstly, a significant increase in research efforts and the involvement of specialists in fish species identification have revealed new or rare fish species that were previously observed very rarely or not mentioned at all for the Barents Sea. Thus, species such as Lütken's eelpout *Lycodes luetkeni*, Paamiut eelpout *L. paamiuti*, checkered wolf eel *Lycenchelys kolthoffi*, and others were found.

Secondly, a number of species have appeared in the Barents Sea due to the warming of the Arctic. As a result, warm water species from more southern regions of the Atlantic, which were previously not observed or were rare here, have begun to be registerd mainly in the southwestern part of the sea - for example, snake pipefish *Entelurus aequoreus*, whiting *Merlangius merlangus*, megrim *Lepidorhombus whiffiagonis*, and others. Additionally, it is necessary to note the appearance of a number of mesopelagic fish species, individual specimens of which have been encountered along the slope of the continental shelf – such as Greenland argentine *Nansenia groenlandica*, Sloane's viperfish *Chauliodus sloani*, and basketwork eel *Diastobranchus capensis* (Wienerroither et al., 2011, 2013).

Thirdly, significant changes have occurred in the taxonomy of several taxa during this period. As a result, the species affiliation of several species has changed (for example, instead of *Liparis gibbus*, *Liparis bathyarcticus* was identified in the Barents Sea; the species *Liparis fabricii* (gelatinous snailfish) was recognized as a complex group consisting of at least three species – *L. fabricii*, *L. koefoedi*, and *L. laptevi*) (Chernova, 2008) or new species have been described, particularly in the genera *Gymnelus* (Chernova, 1999) and *Careproctus* (Chernova, 2005).

Recently the list of ichthyofauna of Barents Sea includes 222 species from 70 families (Dolgov, 2016).

The Kara Sea

Surveys were conducted by PINRO in the Kara Sea in 2006-2007, 2013, and 2019 across the entire sea area, and in 2008-2011 only in the northern part of the sea. During this period, species that were previously not recorded in the Kara Sea were found. Some species, such as Adolf's eelpout *Lycodes adolf*, Lütken's eelpout *L. luetkeni*, archer eelpout *L. sagittarius*, and black-

head strainer snailfish *Rhodichthys melanocephalus*, appear to be rare and were not previously identified by ichthyologists. Other species, such as the mesopelagic ribbon barracudina *Arctozenus risso*, glacier lanternfish *Benthosema glaciale*, spotted lanternfish *Myctophum punctatum*, boreal Greenland halibut *Reinhardtius hippoglossoides*, and beaked redfish *Sebastes mentella*, have appeared due to the warming of the Kara Sea waters (Dolgov et al., 2018). Additionally, some new species have been described, including those from the genus *Careproctus* (Chernova, 2014).

Recently, the list of ichthyofauna in the Kara Sea includes 105 marine and freshwater species from 28 families (Dolgov et al., 2018).

Thus, the current state of biological diversity of the ichthyofauna in the Barents and Kara Seas has significantly increased and generally corresponds to the recent warm climatic period. Under further warming of the Arctic, it is possible that new warm water fish species may appear in these seas.

References

Andriashev, A.P. Fishes of the northern seas of the U.S.S.R. Keys to the fauna of the U.S.S.R. Israel Program for Scientific Translations, 1964, 53, 617 p. 1954.

Andriashev, A.P., and Chernova. N.V. Annotated list of fishlike vertebrates and fish of the Arctic seas and adjacent waters. Journal of Ichthyology, 35(1), 81-123, 1995.

Esipov, V.K. Fishes of the Kara Sea. Leningrad, USSR Academy of Science Press, 145 pp., 1952 (in Russian).

Dolgov, A.V. Species Composition of Ichthyofauna and Structure of Ichthyocenoses of the Barents Sea, Izvestia TINRO, 137, 177–195, 2004. (in Russian)

Dolgov, A.V. Composition, Formation and Trophic Structure of the Ichthyocenes of the Barents Sea. Murmansk, PINRO Press, 393 pp., 2016. (in Russian)

Dolgov, A.V., Novoselov, A.P., Prokhorova, T.A., Fuks, G.V., Prozorkevich, D.V., Chernova, N.V., Sherstkov, V.S., Levitsky, A.L. Atlas of the Kara Sea fish. Murmansk, PINRO Press, 271 pp., 2018. (in Russian)

Karamushko, O.V. Species composition and structure of the ichthyofauna of the Barents Sea. Journal of Ichthyology, 48 (4), 277–291, 2008.

Chernova, N.V. Four New Species of Gymnelus (Zoarcidae) from the Arctic Regions. Journal of Ichthyology, 39 (5), 498–503, 1999.

Chernova, N.V. New data on snailfishes of the shelf of the Arctic seas, Mater. of the International Scientific Conf. "The Nature of the Shelf and Archipelagos of the European Arctic" (Murmansk, 2008), Moscow, GEOS, 8, 377–381, 2008. (in Russian) Chernova, N.V. New Species of Careproctus (Liparidae) from the Barents Sea and Adjacent Waters. Journal of Ichthyology, 45 (9), 689, 2005.

Chernova, N.V. New species of the genus Careproctus (Liparidae) from the Kara Sea and identification key for congeners of the North Atlantic and Arctic. Journal of Ichthyology, 54(10), 757-780, 2014.

Wienerroither, R., Johannesen, E., Dolgov, A., Byrkjedal, I., Bjelland, O., Drevetnyak, K., Eriksen, K.B., Høines, Å., Langhelle, G., Langøy, H., Prokhorova, T., Prozorkevich, D., Wenneck, T. Atlas of the Barents Sea Fishes, IMR/PINRO Joint Report Series, 1-2011, 272 pp., 2011.

Wienerroither, R., Johannesen, E., Dolgov, A., Byrkjedal, I., Aglen, A., Bjelland, O., Drevetnyak, K., Eriksen, K.B., Høines, Å., Langhelle, G., Langøy, H., Murashko, P., Prokhorova, T., Prozorkevich, D., Smirnov, O., Wenneck, T. Atlas of the Barents Sea Fishes based on the winter survey, IMR-PINRO Joint Report Series 2-2013, 220 pp., 2013.

Online database of Antarctic microbial strains catalogue and genome information

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Fungi in Antarctica are concerned about the drastic shrinkage of their habitats due to global warming in recent years. However, only five species and eight strains of fungi isolated from near Syowa Station have been preserved by Japanese microbial culture collections. These fungi are also attracting attention as a new microbial resource because of their specific characteristics at cold temperatures. In this study, we aimed to construct a strain database and a genome database of Antarctic fungi, which have attracted attention as microbial resources but have rarely been preserved in Japanese microbial culture collections.

To construct a strain database, we first attempted to identify 600 strains isolated from samples taken from around Showa Station in Antarctica. The results were as follows. The 584 strains successfully amplified by PCR were sequenced by capillary sequencing.

As a result of DNA sequence analysis, 42 isolates were ascomycetes and could be classified into 15 genera and 15 species. 142 isolates were basidiomycetes and could be classified into 9 genera and 19 species. The remaining 400 isolates were bacterial, so the 16S rRNA, a bacterial marker gene, was amplified by PCR and its gene sequence was determined to confirm that the isolates were bacterial.

The strains identified to fungal species were cultured in yeast peptone dextrose liquid medium (YPD, Difco) and potato dextrose liquid medium at 10°C for 1 week with shaking. Each culture was dispensed into 1 mL of 2 mL cryovials, to which was added 1 mL of 40% glycerol solution containing 10% trehalose, assigned a strain number beginning with NIPR, and stored in a deep freezer at -80°C.

Strain number, species name, sample collection location, and marker gene sequence information are compiled into one database. The database is available on the website of the Bioscience Group of the National Institute of Polar Research (https://www.nipr.ac.jp/biology/).

Some strains were selected, and genomic DNA was extracted from these strains and whole genome sequence analysis was performed using a next-generation sequencer. Gene sequence and gene function predictions were then made from the whole genome sequences. These data are being constructed as a genome database of polar fungi. The genome database is currently in beta version with a password (https://antarcfungi.annotation.jp/), and is scheduled to be released without password on the website of the Bioscience Group, NIPR (https://www.nipr.ac.jp/Biology/) around October 2024.

By releasing the strain and genome databases of these Antarctic fungi, we aim to establish Antarctic fungi as a new microbial resource in the future.

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Microbial diversity and adaptation strategy in the Nunataks of Western Dronning Maud Land, Antarctica.

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To understand the microbial diversity and adaptation strategies in the Antarctic, soil samples were collected from the inland nunataks of Jutulsessen and Ahlmannryggen ranges in Dronning Maud Land, Antarctica. Many bacterial and fungal isolates representing different morphotypes were obtained from different growth media. Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) along with the MALDI BioTyper software, and 16S rRNA domain sequencing techniques were used for identification and classification of bacterial communities of these geographical areas. MALDI-TOF MS phyloproteomic results match with 16S rDNA results more at the genus level than the species level. Out of ninety-six isolates tested in the present study, seventy-five showed good spectral profiles, 13 bacterial isolate's showed known species level identity, and 63 spectral profiles were not matched due to absence of reference spectra in the database. 16S rRNA based known identity of isolates can help in naming unknown MALDI-TOF MS spectra for database implementation. Therefore, the contribution of MALDI-TOF based Antarctic bacterial protein profiles can be used as a powerful tool for microbial identification of environmental samples. ITS and the D1/D2 domain sequencing techniques were also used for characterization of fungal communities in these geographical areas. A total 37 species of culturable bacterial such as Arthrobacter agilis, Acinetobacter baumannii, Arthrobacter flavus, Arthrobacter ginsengisoli, Arthrobacter oxydans, Arthrobacter oryzae, Arthrobacter polychromogenes, Arthrobacter sulfonivorans, Bacillus altitudinis, Bacillus cereus, Bacillus paramycoides, Brevundimonas vesicularis, Brachybacterium rhamnosum, Curtobacterium luteum, Dermacoccus nishinomiyaensis, Dietzia aerolata, Janibacter indicus, Knoellia subterranean, Kocuria palustris, Kytococcus Microbacterium phyllosphaerae, Micrococcus aerolatus, Lysinibacillus sphaericus, yunnanensis, Methylobacterium rhodesianum, Moraxella osloensis, Paracoccus acridae, Pontibacter amylolyticus, Pseudomonas hunanensis, Pseudarthrobacter siccitolerans, Pseudarthrobacter phenanthrenivorans, Rhodococcus aerolatus, Rhodococcus sovatensis, Sphingomonas daechungensis, Sphingomonas sanguinis, Stenotrophomonas pavanii, Staphylococcus gallinarum, Staphylococcus arlettae, and 9 species of fungi such as Candida davisiana, Cosmospora arxii, Geomyces destructans, Lecanicillium muscarium, Memnoniella humicola, Paecilomyces lilacinus, Pseudogymnoascus verrucosus, Phaeophlebiopsis ignerii Thyronectria caraganae were recorded. MALDI-TOF MS and sequencing based identification of the 89 bacterial isolates represented 27 genera and 33 species. Fatty acid methyl esters (FAME) analyses of representative species of each genus have shown predominance of branched fatty acids indicating an adaptation strategy in the Antarctic cold environment. In the non-culturable approach, sequencing of 16S rRNA genes showed unique bacterial community profiles, and dominance of Actinobacteria, Bacteroidetes, Acidobacteria, Firmicutes, Chloroflexi, Planctomycetes, Gemmatimonadetes, Verrucomicrobia and Proteobacteria in the nunatak's samples. To the best of our knowledge, this is the first study on microbial diversity of the Jutulsessen and Ahlmannryggen ranges in Western Dronning Maud Land using both culturable and non-culturable approaches.

Microbial Resilience: Navigating Growth Challenges in Cold Environments

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Antarctic yeast, Glaciozyma antarctica PI12 and Antarctic bacterium, Cryobacterium sp. SO1 have evolved strategies to survive and grow in extreme cold environments although they have optimal growth temperatures above freezing temperatures. Therefore, it would be interesting to determine the mechanisms by which these microorganisms adapted to temperatures above 10 °C while simultaneously thriving in very cold and subzero environments. Hence, this project set out to examine how these microbes respond to colder temperatures at the molecular level. Comparative transcriptome analyses were performed to determine the gene expression patterns of G. antarctica PI12 and Cryobacterium sp. SO1 at optimum and sub-optimal cold temperatures. The results showed that the gene expression patterns of G. antarctica PI12 were dependent on the severity of the cold, with more genes differentially expressed at -12°C than at 0°C than its optimal growth temperature at 12°C. Interestingly, it used nitrite as an alternative terminal acceptor of electrons to minimize disruption of energy production in the cell when oxygen was absent. In contrast, Cryobacterium sp. SO1 upregulated six ribosomal genes, four hydrolases, a clp protease, and a novel YraN family endonuclease related to the programmed cell death (PCD) pathway at 10°C lower than its optimal growth temperature of 20°C, suggesting that the temperature drop was likely lethal to some cells. Three highly upregulated transcriptional regulators were likely key components to regulate genome-wide expression to adapt to the cold among others. Overall, both G. antarctica PI12 and Cryobacterium sp. SO1 had all the necessary genes and well-coordinated mechanisms to adapt to sub-optimal colder or freezing growth temperatures. Their adaptability to cold environments, combined with their preference for growth at temperatures above 10°C, demonstrates their versatility and suggests they will be well-suited to cope with future warming in Antarctica.

References

Teoh, C. P., Lavin, P., Yusof, N. A., González-Aravena, M., Najimudin, N., Cheah, Y. K. and Wong, C.M.V.L. Transcriptomics analysis provides insights into the heat adaptation strategies of an Antarctic bacterium, *Cryobacterium* sp. SO1, Polar Biology, 1-13, 2023.

Wong, C.M.V.L., Boo, S.Y., Voo, C. V.L., Zainuddin, N. and Najimudin, N. A comparative transcriptomic analysis provides insights into the cold-adaptation mechanisms of a psychrophilic yeast, *Glaciozyma antarctica* PI12, Polar Biology, 42, 541 – 553, 2019

Contribution of soil fungal communities to ecosystem multifunctionality in the Arctic

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Increasing studies demonstrated the critical roles of soil microbial communities in ecosystem multifunctionality, the ability of the ecosystem to simultaneously provide multiple functions and services (Manning et al., 2018). However, investigations are scarce in the Arctic tundra, the extremely harsh and vulnerable ecosystem harboring vast carbon reserves. Here, based on the observation in the Sub, Low and High Arctic (Figure 1), we estimated the relationship between multifunctionality and characteristics of soil fungal communities, including α diversity, stability and composition. Fungal communities were detected using high-throughput sequencing by the large subunit (LSU) rRNA gene. Multifunctionality was assessed by both averaging and multiple-threshold approach. We found a positive relationship between fungal diversity and ecosystem multifunctionality across three Arctic regions, and such a relationship was more pronounced in the High Arctic (Figure 2). Moreover, the composition of fungal communities significantly influenced ecosystem multifunctionality, with this relationship being most robust in the High Arctic (Figure 3). Our results highlighted the importance of fungal diversity and community composition in maintaining ecosystem multifunctionality, particularly in more extreme environments like the High Arctic.

Figure 1. Study sites.

Sub Arctic Sub Arctic Low Arctic High Arctic High Arctic A Diversity A Diversity A Diversity

Figure 2. Effects of α diversity (richness) on the number of functions above thresholds. Lines represent the slope between species richness and the number of functions equal to or greater than a threshold value ranging from 5 to 95% of the maximum for each function.

Figure 3. Relationships between fungal community composition and ecosystem multifunctionality in the Sub, Low and High Arctic. Points represent fungal community composition ordinated by non-metric multidimensional scaling (NMDS). Contour lines illustrate the averaged ecosystem multifunctionality, with a shading gradient indicating values.

References

Fanin, N., Gundale, M.J., Farrell, M. *et al.* Consistent effects of biodiversity loss on multifunctionality across contrasting ecosystems, Nature Ecology and Evolution, 2, 269–278, 2018.

Wong, S.K., Kaneko R., Masumoto S., Kitagawa, Ryo., Mori, S.A., and Uchida, M, Functional Gene Composition of Soil Microbial Communities Across a Latitudinal Gradient in the Arctic Region, Polar Data Journal, 6, 1–8, 2022.

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Highly active ice-nucleating particles increased with surface warming over Svalbard

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It is recognized that Arctic low-level clouds occur very frequently over the Svalbard region, where surface warming was anomalously faster than the Arctic average during recent decades. It is generally thought that the clouds are in transition towards a state with more liquid water and less ice due to Arctic warming. On the other hand, our recent field studies in Svalbard have shown that the number concentrations of aerosols capable of nucleating ice (i.e., ice-nucleating particles (INPs)) appear to be enhanced under the influence of certain natural aerosols, such as dust and biological particles, emitted from local/regional sources (Tobo et al., 2019; Freitas et al., 2023). In this presentation, we report on the results of year-round measurements of atmospheric INP number concentrations measured at the Zeppelin Observatory in Svalbard during the Multidisciplinary drifting Observatory for the Study of Arctic Climate (MOSAiC) project period in 2019/20 (Tobo et al., in press). We found that while seasonal variations of the number concentrations of INPs active at temperatures around -30°C were relatively small throughout the year, those active at higher temperatures (i.e., highly active INPs) tended to increase exponentially with rising surface air temperature (SAT) when SAT rose above 0°C and snow/ice-free barren and vegetated areas appeared in the Svalbard region. The ambient aerosol population relevant to the enhanced INP period was largely characterized of dust and biological organic materials most likely originating from local/regional terrestrial sources. Our results suggest that the supply of highly active INPs from Arctic natural sources to the atmosphere could increase in response to surface warming, with implications for the projected cloud-phase transition in the Arctic.

References

Tobo, Y., K. Adachi, P. J. DeMott, T. C. J. Hill, D. S. Hamilton, N. M. Mahowald, N. Nagatsuka, S. Ohata, J. Uetake, Y. Kondo, and M. Koike, Glacially sourced dust as a potentially significant source of ice nucleating particles, Nature Geoscience, 12, 253-258, 2019.

Freitas, G. P., K. Adachi, F. Conen, D. Heslin-Rees, R. Krejci, Y. Tobo, K. E. Yttri, and P. Zieger, Regionally sourced bioaerosols drive high-temperature ice nucleating particles in the Arctic, Nature Communications, 14, 5997, 2023. Tobo, Y., K. Adachi, K. Kawai, H. Matsui, S. Ohata, N. Oshima, Y. Kondo, O. Hermansen, M. Uchida, J. Inoue, and M. Koike, Surface warming in Svalbard may have led to increases in highly active ice-nucleating particles, Communications Earth and Environment, in press.

Characterization of Far-red light harvesting protein complex in an Antarctic alga, Prasiola crispa

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Prasiola crispa, an aerial green alga, forms layered colonies under the severe terrestrial habitats of Antarctica. Since only farred light is available at a deep layer of the colony, *P. crispa* has evolved a molecular system for photosystem II (PSII) excitation using far-red light with uphill energy transfer. We had purified a light-harvesting chlorophyll (Chl)-binding protein complex from *P. crispa* (Pc-frLHC) that excites PSII with far-red light and revealed its unique ring-shaped structure.

Despite the recent report on the structure of Pc-frLHC, which facilitates far-red light absorption and uphill excitation energy transfer to photosystem II, the molecular mechanisms underlying this process remain unclear. Here, we obtained a draft genome sequence of *P. crispa* strain 4113, originally isolated from soil samples on Ongul Island, Antarctica. Notably, 26 genes associated with the light-harvesting Chl *a/b* binding complex (LHC) were identified, including four Pc-frLHC genes, with similarity to a noncanonical LHCI gene with four transmembrane helices. A phylogenetic analysis revealed that Pc-frLHC shares homology with certain LHCI genes found in Coccomyxa and Trebouxia species. This similarity indicates that Pc-frLHC has evolved from an ancestral LHCI gene with four transmembrane helices and branched out within the Trebouxiaceae family. RNA-seq analysis conducted during the initiation of Pc-frLHC gene induction under red light illumination indicated that Pc-frLHC genes were induced independently from other genes associated with photosystems or LHCs. Instead, the genes of transcription factors, helicases, chaperones, heat shock proteins, and components of blue light receptors were identified to coexpress with Pc-frLHC. Those kinds of information could provide insights into the expression mechanisms of Pc-frLHC and its evolutional development.

Figure 1. Photosynthetic mechanisms in Prasiola crispa. (Copyright: Astro Biology Center)

References

Kosugi M, Ohtani S, Hara K, Toyoda A, Nishide H, Ozawa SI, Takahashi Y, Kashino Y, Kudoh S, Koike H, Minagawa J. Characterization of the far-red light absorbing light-harvesting chlorophyll a/b binding complex, a derivative of the distinctive Lhca gene family in green algae. *Front Plant Sci.* 15:1409116, 2024.

Kosugi M, Kawasaki M, Shibata Y, Hara K, Takaichi S, Moriya T, Adachi N, Kamei Y, Kashino Y, Kudoh S, Koike H, Senda T. Uphill energy transfer mechanism for photosynthesis in an Antarctic alga. *Nat Commun.* 14(1):730, 2023.

Kosugi M, Ozawa SI, Takahashi Y, Kamei Y, Itoh S, Kudoh S, Kashino Y, Koike H. Red-shifted chlorophyll a bands allow uphill energy transfer to photosystem II reaction centers in an aerial green alga, *Prasiola crispa*, harvested in Antarctica. *Biochim Biophys Acta Bioenerg*. 1861(2):148139, 2020.

Introduction of a New Project for Terrestrial Biological Observations Conducted from JARE 67 to JARE 69

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The Japanese Antarctic Research Expedition has reached its 66th year. Previous research has revealed that the ice-free areas of the Antarctic continent are inhabited by microscopic, environmentally resilient organisms such as rotifers, mosses, and cyanobacteria. These ice-free areas also contain lakes with various characteristics, including both freshwater and saline lakes. Microbial mats have formed in some of these lakes, and they are also inhabited by rotifers, fungi and various microorganisms. In Antarctic terrestrial ecosystems, these microbial mats are considered the primary biological community.

Recently, a study has reported that the biomass of ephemeral wetlands, which form only during the summer, exceeds that of microbial mats in lakes. However, this study is based on observations from a single wetland and does not provide a comprehensive assessment of wetlands across Antarctica. Previous Antarctic observations have frequently reported large-scale occurrences of rotifers in wetlands. These studies suggest that ephemeral wetlands in Antarctica may serve as significant sites of biological productivity or play an important functional role in linking lakes and terrestrial areas.

In this context, we will conduct a three-year observational project titled "Ecology of Ephemeral Wetlands in an Antarctic Oasis; Aiming to Reveal Environmental Characteristics, Biological Communities, Responses, and Adaptations" This presentation will also outline the details of the project's observational contents.

The climatic and grazing impacts on the reindeer pasture vegetation in the Russian Arctic

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Reindeer husbandry is a traditional land-use practice in the northern regions of the Russian Arctic. Analyzing the dynamics of pasture vegetation has revealed key processes of ecosystem transformation driven by climatic changes and grazing pressure. Currently, we have developed and actively utilize technologies for real-time reindeer pasture inventory using optical satellite data from sources such as Landsat, Sentinel, and MODIS (MOD13Q1.005). Our findings indicate that the initial stages of climate-induced vegetation changes in the European Arctic were most evident during the period from 2000 to 2009, coinciding with a rise in surface air temperatures (SAT) starting in 2000. The European Northeast, in particular, has experienced significant "greening," reflecting increased vegetation productivity during the early stages of this climatic shift. Pastures in this region cover approximately 72.8% of the administrative district area, with reindeer population densities ranging from 0.4 to 2.3 animals per km², averaging 1.2 across all regions.

Figure 1. Trends in Vegetation Transformation During the Initial Stage of Surface Air Temperature Increase (2000-2009) (Elsakov, 2017)

The vegetation of the Northern Timan tundra (Fig. 1A) emerged as one of the most significant "greening" regions within the East European tundra. An analysis of vegetation cover changes in this area was conducted using Landsat, Sentinel, and MODIS satellite imagery from 1985 to 2017, complemented by field observations in 2017-2018. The results showed a 29% increase in reindeer forage capacity from green vegetation between 1985 and 2009, with a 15% increase observed in adjacent areas. After 2007, these changes stabilized.

Interannual trends in NDVI (Normalized Difference Vegetation Index) were confirmed through a combination of medium-resolution MODIS and high-resolution Landsat 5 and 7 images for the same-year intervals. The linear trends in NDVI changes (β), calculated from different sensor data, were comparable and showed a significant positive correlation (r = 0.72, p \leq 0.05). From 2000 to 2009, there was a notable increase in chlorophyll reserves within the phytochromes of the Northern Timan region, amounting to 152 tons. This increase, when expressed as dry green phytomass of low shrubs (including *Betula, Arctous*, and *Empetrum* species), equated to an additional 1.85 centners per hectare. The most significant changes were observed in low shrub lichen tundra communities, where chlorophyll content increased by 1.26 kg/ha and dry green phytomass by 2.51 centners/ha.

The primary factors driving these changes in plant communities include the rise in surface air temperature (SAT) since 2000, particularly during the autumn-winter period, and increased winter precipitation. Additionally, a significant reduction in the reindeer population (a two-fold decrease) likely contributed to the enhanced vegetation productivity in the region.

In contrast, other Indigenous regions (Fig. 1B), which experience more intense reindeer grazing pressure, displayed different trends. In the subarctic and forest-tundra subzones of the Yamal region, satellite imagery from the past 15 years (Fig. 2) revealed a threefold increase in areas with sparse (less than 20%) and less dense (up to 60%) vegetative coverage. Conversely, areas of pasture with high vegetative coverage (80-100%) decreased by 2.5 times, now covering only 25% of the territory. Furthermore, open sand areas expanded by 5.2 thousand hectares (totalling 27.2 thousand hectares, with an average growth rate of approximately 350 hectares per year). The area of disturbed land in 2016 was 9.3 thousand hectares, representing a 6.2-fold increase during the observation period.

A sample of minimal reindeer grazing effects on vegetation was observed in the Novosibirsk Islands archipelago (Fig. 1C). On Kotelny Island, mean annual air temperature values ranged from -17.4°C in 1979 to - 9.9°C in 2020. A green fodder stock model, based on spectral value analysis (Elsakov et al., 2022), was used to

estimate the distribution and dynamics of green biomass during the observation period. From 2000 to 2022, there was a general increase in gross aboveground phytomass stocks across the archipelago.

Figure 2. Changes in Projective Cover (%) of the Yamal Peninsula Tundra based on analyses of satellite image mosaics from Landsat (1988, A), Landsat (2013, B), and Sentinel-2 (2016, C).

The maximum stock values were recorded on Bolshoy Lyakhovsky Island, where phytomass increased from 5.0-6.0 centners per hectare to 8.0-9.0 centners per hectare. The average stock of green phytomass over the observation period showed an increase of 1.23 times on Stolbovoy Island and 2.06 times on Novaya Sibir Island. These changes in green fodder storage could significantly impact the regulation of animal populations in the area, influence changes in seasonal migration routes of wild reindeer, and affect the intensity of element migration.

Figure 3. Distribution of Gross Green Phytomass Stock (centners/ha) at the 2000 (A) and 2022 years (B) of the observation period.

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References

Elsakov, V. Spatial and interannual heterogeneity of changes in the vegetation cover of Eurasian tundra: Analysis of 2000–2016 MODIS data. Current Problems in Remote Sensing of the Earth from Space, 14(6), 56-72. 2017. https://doi.org/10.21046/2070-7401-2017-14-6-56-72

Elsakov, V. V., Zuev, S. M., Mylnikova, T. A. Estimation of the green forage distribution in reindeer pastures based on satellite imagery of different spatial resolution processing. Current Problems in Remote Sensing of the Earth from Space, 19(2), 155-168, 2022. https://doi.org/10.21046/2070-7401-2022-19-2-155-168

Foraging behavior of northernmost population of harbour seals (Poca vitulina)

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Understanding the foraging ecology of animals is essential for their conservation, predicting future population dynamics, and inferring interactions with the ecosystem. Some species are increasing in population due to recent climate warming, and these species are expected to further expand their impact on ecosystems in the coming decades. As a result, it is believed that predation pressure on the prey preferred by these increasing species will intensify, and competition with other species for preferred environments will become fiercer. Therefore, it is necessary to elucidate their detailed foraging ecology. The harbor seal (*Phoca vitulina*) has the widest distribution among pinnipeds. In recent years, the population at its northernmost range (Svalbard, Norway) has been rapidly increasing. This population differs significantly from other populations in terms of environmental characteristics of their habitat, such as the presence of the midnight sun and cold-water temperatures, as well as physical traits such as body size and sexual dimorphism. Consequently, they may exhibit foraging ecology that differs from that of other populations. In this study, we attached video cameras and data loggers to five harbor seals in Svalbard to record their diving and foraging behavior. We will present some of the key findings obtained through fine-scale behavioral analysis.

Fig. 1 Proportion of behavioral categories per hour of a harbour seal (female, 49 kg).

Adélie penguins as a proxy of fine-scale Antarctic sea ice motions

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Adélie penguins are one of the representative top predators in the Antarctic marine eosystem. During incubation period (November to December), they travel handreds km away from their clony to forage in the areas where interfaces coastal and offshore marine ecosystems. Sea ice conditions and its temporal changes in these areas are the important environmental factor for local ocean circulation and most of local marine ecosystem components. While ship-based oceanographic survey is dificult in the spring/early summer, foraging tracks of the Adélie penguins and environmental data along the tracks potentially provide key insights into physical and biological processes in these areas. This study aimed to explore the utility of Adélie penguin foraging tracks to monitor oceanographic conditions, especially sea ice motions. Field study was conducted in Adélie penguin colonies near Syowa station, Antatcitica. We deployed GPS-depth-acceleration data loggers (Axy-Trek, TechnoSmart, Italy) on 5 females in Hukuro Cove in 2017, and 19 females and 13 males in Mamejima in 2023. Amongst them, we successfully recovered enough data from 4 females in 2017, and 14 fmelase and 9 males in 2023, respectively. We interportated the GPS positions every 10 min by Kalman filter. Also, we analyzed the acceleration data to determin body pitching angle and dynamic body acceleration. Subsequently, we classified every 10 min data to four behavioural phases: 1) Nest, 2) Moving on fast ice, 3) Swim, and 4) Surface. Based on the acceleration data, the Surface pahse mainly consisted of resting or preening on the ice, and not of walking/tobboganing or swimming in the very surface layer. Therefore we redarded the Surface phases as Resting on ice. We picked up the tracks during the Rest on ice phase, and analyzed the potential sea ice motions and discuss about the characteristics of surface ocean circulation in the study area.

Survival strategies of algae in the first-year ice of Saroma Ko Lagoon, Hokkaido, Japan: species distribution, shape change, and photosynthetic activity

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Introduction

In recent years, global warming has led to an increase in the first-year ice extent, and further reductions in sea ice extent and thickness are predicted for the near future. Changes in the ice algae community, which is responsible for primary production, will affect the food web and cause changes in the sea ice ecosystem. There is an urgent need to understand the mechanisms that govern the environment in thin sea ice, such as that in Saroma Ko Lagoon, and to study the physiological ecology of the ice algae that inhabit it.

Materials and methods

From February to March 2020, samples were collected from sea ice about 3 km off from Sakaeura on the eastern shore of Saroma Ko Lagoon. The sea ice samples were shaved and collected from the top in every 10 cm depth. Sea water sample was collected from the bottom of sea ice, to compare with these sea ice samples. In a large beaker of filtered seawater was added of the shaved sea ice sample of each layer, gently mixed and ice algae allowed to release in seawater. The released ice algae was allowed to settle overnight under dark condition and the settled ice algal samples were used to photosynthetic measurement and morphological analysis as follows. The photosynthetic characteristics of sea ice algae samples were measured by a pulse amplitude modulation method (WATER-PAM). Each sample was stored in a dark place after fixing with 0.1% low-concentration formalin-based solution until observation. The species composition of the sea ice algae was microscopiccally determined according to the Utermöhl method. Images taken with a phase-contrast microscope were analyzed using software (ImageJ) to determine the diameter of valve and cell length of gardle of *Detonula confervacea* and the number of cells per colony.

Results and discussions

Temperatures at the bottom of the ice remained constant at -1.7° C, near the freezing point, while they varied between -6—1°C with diel fluctuations at the surface layer. By the obtained ice temperature records (*T*, °C), brine salinity (ppt) and its volume fraction of sea ice (ppt) were estimated (Figure 1). The algal communities consisted of more than 95% diatoms. Smaller algal cells were distributed in the upper layer of the sea ice compared to the lower layers. *Chaetoceros* sp., the dominant small-cell species, was evenly distributed throughout the layers. In contrast, *D. confervacea*, the dominant large-cell species, was unevenly distributed in the lower layer, with smaller colony size and cell volume in the upper layer (Figure 2). Carefully collected algal samples showed high photosynthetic quantum yield and acclimation to the light intensities of individual ice layers (Figure 3). This indicates that the algal photosynthetic activity responds to dynamic changes in the ice environment, such as variations in temperature, salinity, and brine space.

In response to the diurnal dynamics of brine, the upper sea ice environment tended to be unsuitable for the growth of ice algae, and as a result, cell division in *D. confervacea* was found to have a vertical difference in morphology related to cell division, such as cell size and colony size. In contrast to the uniform distribution of small-sized ice algae in the sea ice, the large-sized *D. confervacea* were distributed in the lower part of the sea ice. This strongly suggests that the discharge and mixing of brine in the sea ice environment is caused by sedimentation of cells and colonies. From the light-photosynthetic response, the ice algae maintained photosynthetic activity and there was no significant difference in the response pattern according to light intensity. This suggests that the movement and mixing of ice algae is a response to the movement of brine in the sea ice, and shows the accumulation and sedimentation of cell and colony distributions. At first glance, sea ice appears to be a static environment, but it is actually a dynamic 'habitat' for living organisms, and the activity of the ice algae was recorded in response to this dynamic environment by estimating the species and size distribution, and the uniformity of photosynthetic activity.

In the upper layer, there was a clear reduction in cell size and colony size of *D. confervasea*. High photosynthetic quantum yield and the high performance of the ice algal photosynthesis in the entire ice indicated ice algae kept photosynthetic activity in the dynamic changes of the ice environment, such as temperature, salinity, and brine space. *D. confervacea* is a cosmopolitan species inhabiting low-latitude Arctic sea ice regions. The species is expected to have diverse physiological characteristics and may correspond to the rapidly changing first-year ice environment.

Figure 1. Box-and-whisker plots of sea ice temperature (left), salinity (ppt estimated) of brine (middle), and the volume fraction (ppt estimated) of brine to total sea ice (right). Using the ice temperature records obtained from the present study (T, °C), brine salinity (S, ppt) and its volume fraction of sea ice (Bv, ppt) were estimated by applying the two empirical equations,

<i>S</i> =	T/ -	-0.055 (supplied by Nelso	n ar	nd Thor	npson, 1	954)	(1)
n	G.	(40.105/171 + 0.520) (T	1		10	10(7)	

Bv = Sice (49.185/|T| + 0.532) (Frankenstein and Garner, 1967) (2) where *Sice* is the salinity measured from melted sea ice.

Figure 2. Relationship between cell volume and colony size of *D. confervacea* collected from different sea ice depths and seawater. Samples were collected from 5 cm (a), 15 cm (b), 25 cm (c) and 35 cm (d) of sea ice and 45 cm (e) indicated the results of seawater samples below sea ice.

Figure 3. Photosynthetic characteristics of the ice algal communities in each layer of the sea ice. Upper part: Photosynthetic quantum yield of photosystem II (Φ II), the horizontal dotted line shows the maximum value observed in the plankton sample. Middle part: nonphotochemical quenching of chlorophyll fluorescence (NPQ). Lower part: light intensity dependence of relative electron transport rates (rETR). The horizontal dotted line shows the maximum value observed in the 5 cm sample

References

Frankenstein, G., & Garner, R. (1967). Equations for determining the brine volume of sea ice from- 0.5° to -22.9° C. Journal of Glaciology, 6, 943-944.

Nelson, K. H., & Thompson, T. G. (1954). Deposition of salts from sea water by frigid concentration. Technical Report 29 (30 pp.). Seattle, WA: Office of Naval Research.

Effect of delayed sea ice retreat on zooplankton communities in the Pacific Arctic Ocean: A generalized dissimilarity modeling approach

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In the Pacific Arctic Ocean, numerous studies have been conducted on the relationship between sea ice alteration and the community structure of zooplankton. For example, in the northern Bering Sea, the sea ice melt season began a month earlier than usual in 2018, which delayed the spring phytoplankton bloom and resulted in chlorophyll concentrations ten times lower than usual (Huntington et al., 2020). Abe et al. (2020) assessed temporal alterations in the horizontal distribution of zooplankton communities in the Pacific Arctic Ocean using samples collected from 2008–2017. Their generalized linear modeling study revealed a significant positive relationship between the abundance of *C. glacialis* on the Chukchi Shelf slope and the open period of sea ice and integrated mean salinity (IMS) and a significant negative relationship with annual mean temperature. Therefore, studies based on long-term datasets have only analyzed the relationship between sea ice alteration and specific species, and it remains unclear how the entire zooplankton community may alter. In contrast, during the winter of 2020, the Beaufort High disappeared, causing a counterclockwise reversal of the Beaufort gyre, resulting in the eastward transportation of sea ice from the Chukchi Sea to the Beaufort Sea in 2021 (Moore et al., 2022). The increased sea ice concentration may restore the marine ecosystem to its previous state; nevertheless, the precise conditions and mechanisms involved remain unclear in the Pacific Arctic Ocean.

In this study, the 2008–2017 period was defined as "the sea ice retreat year," and its zooplankton community distribution representative was estimated using generalized dissimilarity modeling (GDM). Subsequently, we assessed the effect of delayed sea ice melt on the zooplankton community by comparing the zooplankton community of the sea ice retreat year with that in 2021.

In GDM, numerous satellite parameters significantly affected the zooplankton distribution, with the highest effect during the open-water period and annual primary production (APP) and the lowest in water temperature. The effect of APP and temperature on zooplankton diversity was high around the Bering Strait owing to the advection of Pacific copepods (*Eucalanus bungii*, *Metridia pacifica*, and *Neocalanus* spp.) and synchronized inflow of warm Pacific water. Under significant warming scenarios (Shared Socioeconomic Pathway [SSP]1-2.6 and SSP5-8.5), GDM-based multiple effects predicted that the zooplankton communities in high latitudes will be more affected than those on the southern shelf (northern Bering Sea to southern Chukchi Sea). In 2021, the total abundance across the northern Bering Sea to the Chukchi Sea shelf region was lower than that of the community during the sea ice retreat year. However, certain species (*Limacina helicina* and Pacific copepods) increased locally (northern Bering Sea and Barrow Canyon) because of the increasing volume of Pacific origin water. Even with occasional increases in sea ice concentration, the ecosystem did not simply and

rapidly revert to its previous state, but gradually recovered in the Pacific Arctic Ocean.

References

- Huntington, H.P., Danielson, S.L., Wiese, F.K., Baker, M., Boveng, P., Citta, J.J., et al. Evidence suggests potential transformation of the Pacific Arctic ecosystem is underway. Nat. Clim. Change. 10, 342– 348, 2020.
- Abe, Y., Matsuno, K., Fujiwara, A., and Yamaguchi, A. Review of spatial and inter-annual changes in the zooplankton community structure in the western Arctic Ocean during summers of 2008–2017. Prog. Oceanogr. 186, 102391, 2020.
- Moore, G.W.K., Steele, M., Schweiger, A.J., Zhang, J., and Laidre, K.L. Thick and old sea ice in the Beaufort Sea during summer 2020/21 was associated with enhanced transport. Commun. Earth Environ. 3, 198, 2022.

Figure 3. Horizontal distribution groups identified using a cluster analysis based on principal component (PC) values from generalized dissimilarity modeling (GDM) using the 2008–2017 data set.

OBp3

Nutrient Dynamics of Melt Ponds and Melt Pond Bottom Ice in MOSAiC expedition

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(Gradinger, 2003). Nutrients are an important factor to influence primary production in the Arctic melt ponds (Sørensen et al., 2017). However, nutrient dynamics in melt pond and sea ice under melt pond (pond bottom ice) is not well understood. The melt pond coverage is increasing with global warming (Nicholaus et al., 2012). Therefore, to understand changing Arctic environment, it is getting more important to observe melt ponds and understand nutrient dynamics in the melt ponds, including surrounding environment (e.g. pond bottom ice). In MOSAiC (Multidisciplinary drifting Observatory for the Study of Arctic Climate) expedition, we conducted two melt ponds observations in the central Arctic. We sampled melt pond water and pond bottom ice cores and analyzed nutrients (NO_3^- , NO_2^- , NH_4^+ , PO_4^{3-} , and Si(OH)₄) concentrations.

Comparing with the winter surface seawater condition, nutrients were consumed in the pond water and supplied in pond bottom ice. Decaying particles were observed on the pond bottom. Nutrient maxima occurred at the top of the pond bottom ice (Figure 1). These results suggested that organic matters were produced in pond water, precipitated, remineralized on the pond bottom, and percolated in the porous pond bottom ice. This process is thought to create nutrient maxima at the top of pond bottom ice. Pond water was exchanged with surrounding seawater during observation and PO_4^{3-} and $Si(OH)_4$ increased (Figure 2). Also, snow derived water intruded into pond surface and supplied DIN (= $NO_3^- + NO_2^- + NH_4^+$). In case that melt pond is well mixed after water exchange and snow intrusion, supplied nutrients will support reproduction by algae in the melt ponds. If this nutrient cycle repeated, the nutrient maxima in the pond bottom ice will be enhanced. Furthermore, this specific nutrient distribution, if maintained until refreezing, will affect nutrient distribution in multi-year ice.

Figure 1 Nutrients and sainity vertical distributions in pond bottom ice. The green and red lines refer to MP1 and MP4a, respectively. Green and pink filled areas are parts of melt pond for MP1 and MP4a, respectively.

Figure 2 Nutrients and salinity vertical distributions in pond water. The green and red lines refer to MP1 and MP4a, respectively. The square symbols indicate data obtained on 28th August, and the cross plots indicate data obtained on 14th or 17th September.

References

Gradinger, R., Sea Ice Microorganisms, In Encyclopedia of Environmental Microbiology, 2003.

- Nicolaus, M, Katlein, C., Maslanik, J., and Hendricks, S., Changes in Arctic sea ice result in increasing light transmittance and absorption. Geophysical Research Letters, 39(24), 2012.
- Sørensen, H. L., Thamdrup, B., Jeppesen, E., Rysgaard, S., and Glud, R. N., Nutrient availability limits biological production in Arctic sea ice melt ponds, Polar Biology, 40(8), 1593–1606, 2017.

Spatial distribution and genetic diversity of ice-associated copepods in the Southern Ocean

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The Southern Ocean has a cycle in which 50% of the total area is covered by sea ice in winter, and 80% melts in summer (Parkinson and Cavalieri, 2012). The sea ice is inhabited by bacteria, protozoa, and metazoans introduced from seawater (Ojima et al., 2017). The copepods species *Stephos longipes* and *Drescheriella glacialis* occur in high densities in sea ice in the Southern Ocean, especially in drift ice (Makabe et al., 2022). As drift ice is transported offshore and melts, these copepods are released into the ocean and rapidly incorporated into the food web. Those copepods significantly impact on the ecosystem as much of sea ice melts during the summer. However, previous studies utilized drift ice samples from limited locations with no genetic information. To gain a more comprehensive understanding of sea ice copepods, we analyze the population density and genetic diversity of *S. longipes* and *D. glacialis* within sea ice collected from diverse locations in the Indian sector of the Southern Ocean.

From January 2019 to March 2020, sea ice (drift ice) pieces were collected from a total of 20 stations in three areas of the Indian Sector of the Southern Ocean : Lützow-Holm Bay (3 stations), off Cape Darnley (3 stations), and off Totten Glacier (14 stations). *S. longipes* and *D. glacialis* were sorted from Lugol's iodine-fixed sea-ice meltwater under a stereomicroscope, and the count of individuals at each life stage (nauplii, copepodite, and adult) was recorded for samples collected from December 2019 to March 2020. Based on the counting results, the population densities of both species were calculated for each station. For genetic analysis, 5 to 10 individuals of each species were randomly selected per observation area, and total DNA was extracted from each using a modified Lysis buffer method (Kobayashi et al., 2022). PCR was then employed to amplify partial sequences of the mitochondrial cytochrome b gene (*mt-cytb*) and 18S rRNA gene (*18SrRNA*) using this DNA as a template. The nucleotide sequences of both genes were used to create maximum likelihood phylogenetic trees. In this study, *mt-cytb* was used to confirm the presence of intraspecific genetic polymorphisms, while *18rRNA* was used for species identification. In addition to these drift ice derived individuals, we also conducted similar genetic analyses of seawater-derived individuals from the Adélie Land, Davis Station, and Mawson Coast of East Antarctica.

The highest population densities of *S. longipes* were observed off Totten Glacier $(4.9 \times 10^4 \text{ ind./m}^3)$, followed by Lützow-Holm Bay $(3.2 \times 10^4 \text{ ind./m}^3)$ and off Cape Darnley $(1.2 \times 10^4 \text{ ind./m}^3)$. For *D. glacialis*, the highest densities were recorded off Totten Glacier $(9.6 \times 10^3 \text{ ind./m}^3)$, followed by off Cape Darnley $(2.7 \times 10^3 \text{ ind./m}^3)$ and Lützow-Holm Bay $(2.2 \times 10^2 \text{ ind./m}^3)$. *D. glacialis* was rarely observed in Lützow-Holm Bay. Overall, *S. longipes* had higher population density. In terms of differences due to the location of stations, the drift ice collected off Totten Glacier tended to have higher population densities for both species than those collected near the continental shelf, away from the coast. Among the offshore Totten Glacier samples, drift ice collected near Dalton Polynya had lower population densities. In Lützow-Holm Bay, the population densities tended to be higher for drift ice at stations farther from shore than for ice in the near-shore sea ice area. However, for the area off Cape Dunley, the sea ice near the coast had a higher population density.

Genetic analysis revealed that *S. longipes* exhibited monophyly in both *mt-cytb-* and *18SrRNA*-based phylogenetic trees, with no identified intraspecific genetic polymorphisms. In contrast, *D. glacialis* demonstrated monophyly in the *18SrRNA* based phylogenetic tree and also exhibited divergence into two lineages on the *mt-cytb-*based phylogenetic tree, suggesting that the existence of intraspecific genetic polymorphisms in *D. glacialis*.

References

Kobayashi, et al. Bull. Plankton Eco-Eng. Res. 2, 56-65. 2022. Makabe, R., et al. Polar Biology. 45, 749-762. 2022. Ojima, et al. Polar Science. 12, 19–24. 2017. Parkinson, et al. Cryosphere. 6, 871-880. 2012.

Classification of larvae of paralepidid fish Notolepis spp. in the Southern Ocean

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Notolepis coatsorum (Family: Paralepididae) is a mesopelagic fish widely distributed from the continental slope of the Antarctica to the vicinity of the Antarctic Front, serving as prey for various top predators. Another species of the genus, *N. annulata*, is known from the sub-Antarctic region, but there are few collection records and the distribution patterns of this species remain unknown. The larvae of both species are distinguishable by characteristics such as the position of pigmentation on the peritoneum and the number of myomeres, but their external morphology is very similar, making identification difficult in the early-stage larvae. This study attempted species identification of *Notolepis* larvae collected from the Indian Ocean sector of the Southern Ocean using DNA barcoding. DNA was collected from the skin, extracted using the Chelex method, and the mitochondrial COI region (approximately 650 bp) was amplified by PCR and sequenced. As a result, three types of sequences were identified, with 97.2-96% homology between types. Three types of data were also registered as *N. coatsorum* in the database, and they corresponded to each of them. N. annulata was not registered in the Database, but one of the three types was similar to the characteristics described as N. *coatsorum*, revealed no morphological differences. The information obtained in this study was organised with the database information to examine the distribution patterns, but no clear differences were observed among the three types.

南大洋に分布する Notolepis 属魚類(ハダカエソ科)仔魚の分類

ハダカエソ科の Notolepis coatsurum は、南大洋の大陸斜面域から南極前線付近まで広く分布し、様々な高次捕食者の餌と なる中深層性魚類である。Notolepis 属にはもう一種 N. annulata が亜南極域から知られているが、採集記録が少なく分布様 式については不明な点が多い。両種の仔魚については、腹腔の色素の位置や筋節数などで区別できるとされているが、外 部形態はよく似ており、サイズによっては同定が困難である。本研究では、南大洋インド洋セクターで得られた Notolepis 属 仔魚について、DNA バーコーディングによる種同定を試みた。DNA は体表から採取したのち、キレックス法で抽出した。 PCR 法でミトコンドリア COI 領域(約 650 bp)を増幅しシーケンスによって配列を得た。NCBI database および Bold system を 使って検索を行い、97%以上の相同性をもって種判別した。分析した Notolepis 属の配列には3 タイプが存在し、タイプ間の 相同性は 97.2~96%であった。Database の中にも N. coatsorum と登録されているデータが 3 タイプあり、それぞれに該当し ていた。N. annulata は Database に登録が無かったが、3 タイプのうち 1 タイプは、形態から N. annulata として記載されてい る仔魚の特徴によく似ていた。一方、N. coatsorum とされる 2 タイプについて、プロポーションなど詳細に比較したが形態的 な差異は認められなかった。本研究で得られた、サンプルと database の情報を整理し分布様式について検討したが、3 タイ プに明瞭な差異は認められなかった。

Summer microplankton and mesozooplankton communities in surface waters of three adjacent marine areas of northwestern Greenland: What determines area-specific differences?

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In Greenland, the melting of glaciers has increased due to global warming, leading to a greater intrusion of glacier meltwater into the fjord. The impact of glacier meltwater on the marine ecosystem may vary across sites due to geographical and environmental differences. However, limited studies have been conducted between adjacent fjords in the same geographical area. This study was conducted on the summer microplankton and mesozooplankton surface communities of three adjacent marine areas: Bowdoin Fjord, Inglefield Bredning, and Robertson Fjord in northwestern Greenland. Through this comparison, we aim to identify the environmental factors that control the planktonic communities in these three areas of northwestern Greenland.

Between July 27 and August 11, in both 2016 and 2022, we collected sea surface water samples, towed a horizontal plankton net at the sea surface, and performed CTD casts at 7–13 stations in each area. The seawater samples were preserved and concentrated, and then the microplankton organisms were identified and counted. The plankton net samples were also preserved, and taxonomic identification and counting were conducted for mesozooplankton. A Max-t test assessed regional differences in environmental parameters, microplankton, and mesozooplankton abundance between the three regions. The parameters influencing the abundance of mesozooplankton were analyzed using a decision tree.

The Bowdoin Fjord surface waters were characterized by a relatively high temperature, low salinity, high turbidity, and high Chl. *a*. Ciliates were the dominant microplankton, and barnacle larvae dominated the mesozooplankton community. This hydrological environment was influenced by the inflow of highly turbid meltwater from the land-terminating glacier and by upwelling plumes near the marine-terminating glacier. It has been reported that meltwater from the bottom of the marine-terminating glacier supplies iron, which combines with nutrients in the deep water and promotes phytoplankton growth in Bowdoin Fjord. However, the highly turbid water inhibits light penetration, resulting in low primary production. Bowdoin Fjord was the most nutrient-rich of the three fjords, and the favorable food and benthic environment supported the growth and reproduction of barnacles, leading to the dominance of barnacle larvae in the zooplankton community.

The Inglefield Bredning area showed a high water temperature, medium salinity and turbidity, and low Chl. *a*. The zooplankton was dominated by the jellyfish *Aglantha digitale*. Although Inglefield Bredning comprises the deepest habitats among the three compared areas, the relatively warm subsurface Atlantic waters reach it directly from the open ocean. The jellyfish *A. digitale* is known to prey on small copepods. Many small copepods, such as *Acartia spp.*, were also present in this area and thus constituted a suitable feeding environment for *A. digitale*. Decision tree analysis also showed that water temperature was a determining factor for the jellyfish population, with larger populations under warm conditions. Accordingly, the total biomass of *A. digitale* is anticipated to increase with global warming. These results suggest that *A. digitale* was dominant because the Inglefield Bredning is a favorable feeding environment with relatively high water temperatures.

In contrast, the Robertson Fjord showed low water temperatures, high salinity, low turbidity, and a moderate level of Chl. *a*. Among the microplankton, dinoflagellates were the dominant species, and copepod nauplii were dominant among the mesozooplankton. Robertson Fjord is the northernmost of the three areas and is primarily influenced by oceanic water of Arctic origin. Compared to the other two marine environments, Robertson Fjord was the least affected by glacial meltwater and had the most oligotrophic environment. The low turbidity and high visibility of Robertson Fjord provide adequate conditions for visual predatory larvae to feed on the dominant nauplii larvae. The dominant nauplii larvae in Robertson Fjord were N5 and N6 of the large oceanic copepod *Calanus hyperboreus* spawned in spring, based on the reported relationships between water temperature and growth and body size.

Currently, Greenland glaciers are melting due to global warming, and freshwater inflow into the ocean through the fjords is increasing. The increase in freshwater inflow due to glacial meltwater is expected to result in a highly turbid environment. Global warming is also anticipated to cause the warm Atlantic water to expand northward. Considering these factors, the oceanographic characteristics and processes may intensify in Bowdoin Fjord and Inglefield Bredning, while they may weaken in Robertson Fjord, in the future.

Population genetic structure of Mosses from the Langhovde, Skarvsnes and Skallen, East Antarctica, analyzed by genotyping

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All plant species have limits to their distribution, and their populations demarcate margins and demonstrate the end-point of adaptation to environmental changes (Crawford 2008). In many cases, a failure to reproduce may be a more common response to environmental limitations than a failure to grow, probably because reproductive success requires more than just the development of viable seeds. Terrestrial plant populations located at the margins of species' distribution often display reduced sexual reproduction and increased reliance on asexual reproduction (e.g. Eckert 2002, Pigott 1981). For example, at the northern limits of *Betula glandulosa*, which can reproduce both sexually and asexually, less than 0.5 % of the seeds are viable and populations are maintained by asexual reproduction (Weis *et al.* 1993).

In a view of generally acquired plant physiology, any polar region (Antarctic regions) is not a habitable rather very stressful zone for most of plants. This is due to its low temperature, long time snow covering, and direct solar irradiation. To overcome these environmental stresses, Antarctic plants are forced to shorten their time for growing and propagation to the moderate season during each year. Eight species of bryophytes have been observed growing around Syowa Station in Antarctica, but only three of them has been observed to form sporophytes. This suggests that the Syowa Station area is near the limit of bryophyte distribution and sexual reproduction. However, it is difficult to extract the amount of DNA needed for conventional DNA analysis from Antarctic bryophyte samples, and since no studies have yet examined the genetic diversity of bryophytes around Syowa Station to clarify their reproductive mode.

We used the MIG-seq technique to examine the genetic diversity within the *Pottia heimii* and *Bryum pseudotriquetrum* populations around Syowa Station from a smaller amount of DNA samples. *Pottia heimii* is one of the sporophyte forming species, and no sporophytes have ever been found on *Bryum pseudotriquetrum* in this area.

In *Pottia heimii*, the heterozygosity within the population was low and the degree of relatedness was high. Fst was also very low, confirming little differentiation. This suggests that although *Pottia heimii* forms sporophytes, it is unlikely that this species successfully reproduce sexually. In *Bryum pseudotriquetrum*, the genetic diversity was examined among three regions, Langhovde, Skarvsnes and Skallen. Some populations growing in Langhovde had slightly higher Fst, moderately differentiated, and were less closely related in heterozygosity. However, the Fst tended to be relatively low in Skarvsnes and Skallen compared to Langhovde. A comparison of genetic diversity within populations by geographic location revealed low Fst and little differentiation between Skarvsnes and Skallen. The moderate differentiation between Langhovde and the two sites mentioned above suggested that differentiation may have occurred in different habitats. These results may suggest that the genetic diversity of Anterctic mosses is low, even in the case of sporophyte forming species.

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References

Crawford, R. M. M., Plants at the margin. Ecological limits and climate change, Cambridge University Press, Cambridge, 1-494, 2008.

Eckert, C. G., Loss of sex in clonal plants, Evol. Ecol., 15, 501-520, 2002.

Pigott, C. D., Nature of seed sterility and natural regeneration of *Tilia cordata* near its northern limit in Finland, Ann. Bot. Fennici, 18(4), 255-263, 1981.

Weis, I. M. and L. A. Hermanutz, Pollination dynamics of arctic dwarf birch (*Betula glandulosa*; Betulaceae) and its role in the loss of seed production, Am. J. Bot., 80(9), 1021-1027, 1993.

Expression and analysis of enzymes for carbazole metabolism from strain BS-19

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Heterocyclic compounds including carbazoles and polycyclic aromatic hydrocarbons (PAHs) are found in petroleum and other fossil fuels and are deposited into the environment by human activities. These compounds are considered to be mutagenic, carcinogenic, and teratogenic. Therefore, monitoring and removal of these substances is necessary. Bioremediation has a low environmental impact and is expected to be one of the most promising environmental remediation methods in environments such as Antarctica and in environments where the introduction of new plants and animals is severely restricted. In order to utilize bioremediation effectively, bacteria strains which can degrade heterocyclic compounds and PAHs have been studied by researchers .

Sphingobium sp. strain BS-19 was the first report of a bacterium isolated from Antarctica that could degrade heterocyclic compounds such as carbazole and PAHs (Kenta Sato et al. 2023). Therefore, strain BS-19 has the potential to contribute to bioremediation in Antarctica. During carbazole metabolism, a colored metabolite was produced after *meta*-cleavage reaction. The enzymes responsible for this reaction was identified as *meta*-cleavage enzyme. This charactaristics may be used to detect PAHs and related compounds for environmental monitoring applications. This study aims to express and evaluate the *meta*-cleavage enzyme encoded by *carBa* and *carBb* genes located in the carbazole degradation gene cluster (Figure 1).

The *meta*-cleavage enzyme (*CarBa,Bb*) gene of strain BS-19 was amplified by PCR and inserted into the pUC18 plasmid using restriction enzymes to create expression plasmid pUC18_CarBa_Bb. The pUC18_CarBa_Bb expression was confirmed by the following resting cell reaction. *E. coli* strain DH5 α cells harboring expression plasmids were cultivated in 100 ml of LB medium at 30°C. When OD₅₅₀ reached 0.8, isopropyl-b-D-thiogalactopyranoside (IPTG) was added to a final concentration of 0.5 mM. After that incubated another 5 hours and the cells washed twice and suspended by PBS (-). The suspended cells were added 4-methylcatechol to a final concentration 0.1% (v/v) and the rate of 4-methylcatechol reduction was measured using GC-FID. 8 hours of resting cell reaction degraded about 25% of 4-methylcatechol.

In the future, the ability of *meta*-cleavage enzymes in other substrates will be evaluated, as well as the expression of other carbazole-degrading genes.

Figure 1. Structure of carbazole degradation gene cluster of strain BS-19 *meta*-cleavage enzyme is encoded by *CarBa* and *CarBb*

References

Kenta Sato. Seiryu Take. Siti Aqlima Ahmad. Claudio Gomez-Fuentes. and Azham Zulkharnain, Carbazole Degradation and Genetic Analyses of *Sphingobium* sp. Strain BS19 Isolated from Antarctic Soil, Sustainability, 15(9), 7197, 2023.

Enzymatic activity analyses of di(2-ethylhexyl)phthalate (DEHP) degrading bacterium strain HP5 isolated from Antarctic soil

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The destruction of ecosystems due to the accumulation of microplastics in living organisms is an global problem. DEHP is a type of plasticizer mainly used as an additive in the processing of plastics. DEHP is easily leaked into the environment, but even when leached into the environment, it does not decompose naturally and has been reported to cause carcinogenic and reproductive dysfunction. Bioremediation, which uses the metabolism of bacteria, can be utilized to remove DEHP from the environment. While bioremediation has the advantage of low environmental impact and low cost, it also has several drawbacks, such as being dependent on environmental conditions and levels of contamination. Therefore, this study aims to analyze the characteristics of DEHP-degrading bacteria *Rhodoccocus* sp. strain HP5 isolated from Antarctic soil and develop bioremediation methods using this strain.

Strain HP5, a bacterium isolated by screening from Antarctic soil-derived flora, was used to determine the degradation rate in the presence of high concentrations of DEHP, identify enzymes required for degradation, observe morphological changes, and predict genes related to DEHP degradation. The final concentration of DEHP was 200 mg/L, 1000 mg/L L. MSM (minimum salt medium) was adjusted to achieve a final concentration of DEHP of 200 mg/L, 1000 mg/L. HP5 strains were inoculated and incubated at 30°C, 120 rpm for 15 days, and the degradation rate and viable bacterial counts were determined every 3 days by HPLC and CFU counting method. For hydrolytic enzyme activity evaluation, 8 mL of culture medium was taken every other day, and the supernatant was centrifuged to remove impurities by filtering; 1× PBS, sample, and Triton X-100 kept at 30°C for 1 hour were mixed, and substrate was added and shaken at 30°C and 140 rpm for 30 minutes. The amount of pNP released was measured using a spectrophotometer at a wavelength of 405 nm. In this study, 1 U was defined as the amount of enzyme releasing 1 μ mol of pNPs per minute. 1 U of dehydrogenase was collected and suspended in MilliQ water as a sample for evaluation of 2 days. Methanol was added to extract the dye, and the dye was measured using a spectrophotometer at a wavelength of 485 nm. For prediction of genes related to DEHP-degradation, primers were created from genes of hydrolytic enzymes involved in

DEHP degradation reported in the previous literature and PCR was performed. HP5 strains were incubated with glucose and DEHP at 200 mg/L or 1000 mg/L for 3 days and observed using SEM.

Although the HP5 strain did not have a high degradation rate in the presence of high concentrations of DEHP, the amount of degradation was more than twice that in the presence of low concentrations of DEHP. The amount of hydrolytic enzyme produced in vitro was also higher in the presence of high concentrations of DEHP. This was considered to indicate that strain HP5 is a suitable bacterium for degradation in high concentrations of DEHP. However, the decrease in the number of viable bacteria and the small size of the HP5 strain at high concentrations of DEHP indicated that high concentrations of DEHP inhibited the growth of the HP5 strain. In addition, it is thought that β -oxidation of PAE with long side chains is preferentially performed because the side chains generated by hydrolysis are toxic to bacteria , and that the toxicity of metabolites such as 2-ethyl-1-hexanol generated by hydrolysis inhibits or inhibits the growth of HP5 strains.

References

Paola Pocar, Nadia Fiandanese, Anna Berrini, Camillo Secchi, Vitaliano Borromeo, Maternal exposure to di(2-

ethylhexyl)phthalate (DEHP) promotes the transgenerational inheritance of adult-onset reproductive dysfunctions through the female germline in mice, *Toxicology and Applied Pharmacology*, 322, 113121, 2017

National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, DEHP: Genotoxicity and potential carcinogenic mechanisms, *Mutation Research*, 751, 82157, 2012

National Institute of Technology and Evaluation. Biotechnology. Nite.

https://www.nite.go.jp/nbrc/safety/bioremediation.html #

Fangyuan Chen, Xuli Li, Yiqie Dong, Jiahao Li, Yixin Li, He Li, Lei Chen, Min Zhou, Haobo Hou, Biodegradation of phthalic acid esters (PAEs) by Cupriavidus oxalaticus strain E3 isolated from sediment and characterization of monoester hydrolases, *Chemosphere*, 266, 129061, 2021

Rhodopsin-containing Bacteria Isolated from Ellesmere Island in the High Canadian Arctic

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Recent scientific breakthroughs have unveiled the intriguing role of rhodopsin, a specialized protein, in microbial life. For example, cells containing the proton pumping rhodopsin can function similarly to solar panels by harnessing light energy to generate proton motive force across cells. This electrochemical gradient then serves as a power source for ATP production and supports energy-demanding processes, including motility, organic matter degradation, and cell survival during challenging conditions. Recent findings of proteorhodopsin from the marine environment showed that bacteria can capture and use light energy, akin to photosynthetic organisms like plants and plankton. Some studies even suggest that rhodopsin-based phototrophy is a primary means of capturing solar energy in the surface ocean, rivaling the energy production of chlorophyll-a. Rhodopsin-bearing bacteria, cyanobacteria, eukaryotes, and viruses have since been identified in various environments. Together with recent advancements in molecular methods, different types of rhodopsin ion pumps have also been discovered including sodium (Na^+) and chloride (Cl^-) pumps. Despite its prevalence in aquatic environments, the distribution of rhodopsin in polar regions remains largely unexplored. We successfully isolated three rhodopsin-possessing bacterial strains from a cryoconite (2 strains) and a moss ball (1 strain) collected from Ellesmere Island in the Canadian High Arctic. There strains were isolated and grown on a 1/10-strength Reasoner's 2A (R2A) agar (pH 7.0, Difco) after serial dilution with 1X PBS (-) buffer (pH 7.3). The 16S rRNA gene sequences showed that strain 251, strain 252, and strain 141 were closely related to the genera Cryobacterium, Pedobacter, and Hymenobacter, respectively. The draft genome sequences of Pedobacter sp. strain 252 and Hymenobacter sp. strain 141 were typical of the H^+ ion-transport Proteorhodopsin clade. Interestingly, the draft genome sequence of Cryobacterium sp. strain 251 showed that the strain possesses four different rhodopsin-related genes. Two copies of the genes were closely related to the Heliorhodopsin cluster, while the other two were related to the ion transport (H^+) rhodopsin groups, namely, Xanthorhodopsin and Proteorhodopsin. The main functions of the Heliorhodopsin clusters are still relatively unknown but a bacterium possessing four different rhodopsin genes is relatively rare in the environment. Future studies will focus on the comparative genome between Cryobacterium sp. strain 251 and other multiple rhodopsin-possessing bacteria as well as determining the conditions that lead to the expression of the rhodopsin genes in Cryobacterium sp. strain 251 to help explain the adaptations that may help the Arctic bacteria to utilize different wavelengths and light sources that are available.

Preliminary identification of *Globisporangium* species from decayed *Ulva* sp. in Ny-Ålesund, Spitsbergen Island, Norway

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Globisporangium spp. is a plant-parasitic oomycete with a global distribution, including the polar regions (Tojo et al. 2001). *Ulva* spp., commonly known as edible seaweeds, are widely distributed in the littoral zones, including the northern districts of Norway (Roleda et al. 2021). In July 2024, isolates of an oomycete species were obtained from decayed *Ulva* sp. in Ny-Ålesund, Spitsbergen Island, Norway. These isolates produced zoospores following the formation of vesicles from germinating globose sporangia. Based on these morphological traits, the isolates were identified as belonging to the genus *Globisporangium*. No oospore production was observed in the isolates studied. Although both *Globisporangium* and *Ulva* are cosmopolitan genera, their co-existence has not been previously documented. This study was conducted to elucidate the ecological interactions between *Globisporangium* and *Ulva* species in polar environments. Preliminary results indicate that the *Globisporangium* sp. proliferated on decayed *Ulva* sp.

Figure 1. Site where Globisporangium species from decayed *Ulva* sp. in Ny-Ålesund, Spitsbergen Island, Norway. Arrowheads show decayed *Ulva* sp.

Figure 2. Morphology of sporangia of *Globisporangium* species isolated from decayed *Ulva* sp. in Ny-Ålesund, Spitsbergen Island, Norway. Arrowhead shows vacant sporangium with elongation tube after producing zoospores. Bar: 20 μ m

References

- Roleda, M. Y., Lage, S., Aluwini, D. F., Rebours, C., Brurberg, M. B., Nitschke, U., Gentili, F. G. Chemical profiling of the Arctic sea lettuce *Ulva lactuca* (Chlorophyta) mass-cultivated on land under controlled conditions for food applications. Food Chemistry 341, 127999, 2021
- Tojo, M., Fujii, N., Yagi, H., Yamashita, Y., Tokura, K., Kida, K., Hakoda A., Herrero, M. L., Hoshino, T. Uchida, M.. Identification and isolation pattern of *Globisporangium* spp. from a *Sanionia* moss colony in Ny-Ålesund, Spitsbergen Is., Norway from 2006 to 2018. Microorganisms 9, 1912. 2021

Investigation of phenanthrene degradation pathway and low-temperature biodegradation using Antarctic bacterium *Pseudarthrobacter* sp. BS28

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Phenanthrene (PHE) is a class of polycyclic aromatic hydrocarbons (PAHs). PAHs and their related compounds are genotoxic, carcinogenic, mutagenic, teratogenic, and persistent, and pose a serious health risk to organisms when released into the environment without treatment (Venkatraman et al., 2024). PAHs contamination is widespread globally, and PHE have been detected even in Antarctica, the least populated continent in the world. Several degradation pathways for PHE by bacteria have been reported. In general, PHE is degraded to 1-hydroxy-2-naphthoic acid (1H2N), followed by phthalate or salicylic acid pathway, and then enters the TCA cycle. Li et al. (2024) reported that *Pseudarthrobacter* sp. L1SW degrades PHE by phthalic acid pathway. PHE-degrading bacterium, *Pseudarthrobacter* sp. BS28, was isolated from soil near the Bernard O'Higgins Riquelme base. To understand the degradation pathway of strain BS28, the ability to utilize 1H2N and phthalic acid were investigated. Strain BS28 ability to degrade PHE at low temperature was also investigated for bioremediation in extremely cold regions such as Antarctica.

MSM (minimum salt medium) was prepared so the final concentration of PHE reached 500 mg/L. After inoculation of strain BS28, the culture was incubated at 15°C and 135 rpm for 7 days. After incubation, the culture medium was centrifuged and strain BS28 was washed in sterile phosphate buffer. The culture was resuspended in sterile phosphate buffer and the optical density at 600 nm was adjusted to 0.8. This was used as the pre-culture solution. First, Experiments on the degradation of PHE by mixing PHE with compounds reported as metabolic intermediates (1H2N and phthalic acid) were performed in glass test tubes. The preculture solution was inoculated into test tubes and incubated at 15°C and 135 rpm. Residual PHE in the test tubes was extracted with ethyl acetate and the residual concentration was determined by gas chromatography hydrogen flame ionization detector (GC-FID, GC 2014, Shimadzu, Japan). PHE degradation experiments were conducted at 5°C with an initial concentration of 500 mg/L of PHE. The preculture solution was inoculated into test tubes and incubated at 5°C and 135 rpm. Residual PHE in the test tubes of 500 mg/L of PHE. The preculture solution was inoculated into test tubes and incubated at 5°C and 135 rpm. Residual PHE in the test tubes were extracted with ethyl acetate and the residual concentration was inoculated into test tubes and incubated at 5°C and 135 rpm. Residual PHE in the test tubes were extracted with ethyl acetate and the residual concentration was inoculated into test tubes and incubated at 5°C and 135 rpm. Residual PHE in the test tubes were extracted with ethyl acetate and the residual concentration was inoculated into test tubes and incubated at 5°C and 135 rpm. Residual PHE in the test tubes were extracted with ethyl acetate and the residual concentration was determined by GC-FID.

When cultured with a mixture of PHE and 1H2N, neither PHE nor 1H2N showed any decrease. In the growth experiment conducted at the same time as this experiment, the number of viable bacteria did not increase after 3 days of incubation, suggesting that strain BS28 is not capable of utilizing 1H2N or 1H2N had a growth inhibitory effect. After incubation with a mixture of PHE and phthalic acid, neither PHE nor phthalic acid showed any reduction. Strain BS28 was capable of degrading PHE at 5°C. After 28 days and the amount of PHE remaining decreased to 56.76%. This strain also showed an increase in the number of viable bacteria. Generally, when bacteria degrade PAHs by enzymes, higher temperatures tend to increase the activity of the enzymes and their ability to degrade PAHs. Strain BS28 was incubated at 5°C for 28 days and degraded 43.2% of 500 mg/L PHE. However, no significant degradation was observed when incubated at 30°C. This may be evidence of the cold adaptability shown by strain BS28 through PHE degradation. Many cold-adapted bacteria have been reported previously. Wang et al. (2021) showed that the biodegradation of 5 mM p-nitrophenol by *Psychrobacter* sp. ANT206 cultured at 16°C was 92.47%, higher than that at 20°C (80.59%).

In the future, the metabolic intermediates of PHE will be identified using GC-MS and the degradation pathway of strain BS28 will be predicted.

References

Li, J., Peng, W., Yin, X., Wang, X., Liu, Z., Liu, Q., Deng, Z., Lin, S., and Liang, R., Identification of an efficient phenanthrene-degrading *Pseudarthrobacter* sp. L1SW and characterization of its metabolites and catabolic pathway. *Journal of Hazardous Materials*, 465, 133138, 2024.

Venkatraman, G., Giribabu, N., Mohan, P. S., Muttiah, B., Govindarajan, V. K., Alagiri, M., Abdul Rahman, P. S., and Karsani, S. A., Environmental impact and human health effects of polycyclic aromatic hydrocarbons and remedial strategies: A detailed review. *Chemosphere*, *351*, 141227, 2024.

Wang, Y., Hou, Y., Wang, Q., and Wang, Y., The elucidation of the biodegradation of nitrobenzene and p-nitrophenol of nitroreductase from Antarctic psychrobacter sp. ANT206 under low temperature. *Journal of Hazardous Materials*, *413*, 125377, 2021.

Seasonal changes in photosynthetic activity and their relation to the phenology of deciduous plants in the High Arctic, Svalbard

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Understanding the photosynthetic characteristics is essential for predicting the impact of climate change on carbon cycling in Arctic terrestrial ecosystems. However, despite their importance to net primary production, few studies have investigated seasonal changes in photosynthetic activity during the growing season (Muraoka et al., 2002; Starr et al., 2008). In this study, we investigated seasonal changes in photosynthetic activity and their relation to the phenology of deciduous plants in the High Arctic, Svalbard.

Our study site was located at Nybyen, Longyearbyen, Svalbard, Norway (78°12'N, 15°35'E). The three selected plant species are common deciduous plants in Svalbard: *Salix polaris* (dwarf shrub), *Oxyria digyna* (forb), and *Bistorta vivipara* (forb). To investigate photosynthetic activity, we used a portable photosynthesis system (LI-6400, LI-COR). The photosynthetic rates were determined at light saturation (PPFD = 750 μ mol m⁻² s⁻¹) and approximately 10°C. We conducted these measurements every three to five days from July 7 to August 9, 2024.

Both *S. polaris* and *O. digyna* maintained their high photosynthetic rates until July 25, after which these rates began to decrease synchronously with leaf color changes (from green to yellow). In contrast, *B. vivipara* maintained high photosynthetic rates for a longer period than *S. polaris* and *O. digyna* did (at least until August 5). However, the signs of a decline in photosynthetic rates appeared to be synchronized with changes in leaf color, similar to the previous two plant species. Our results indicate that although interspecific differences exist in the duration of high photosynthetic rates, seasonal changes in photosynthetic activity have a strong relationship with phenology. Moreover, since leaf color changes occurred irrespective of air temperature changes (Fig. 1), internal factors might determine phenological changes in the leaves of these deciduous plants.

Fig. 1. Hourly air temperature in Longyearbyen from July 1 to August 31, 2024 (https://seklima.met.no/). The range outlined in red shows the period when changes in leaf color began to be observed.

References

- Muraoka H., Uchida M., Mishio M., Nakatsubo T., Kanda H., Koizumi H. (2002) Leaf photosynthetic characteristics and net primary production of the polar willow (*Salix polaris*) in a high arctic polar semi-desert, Ny-Ålesund, Svalbard. Can. J. Bot. 80:1193-1202.
- Starr G., Oberbauer S.F., Ahlquist L.E. (2008) The photosynthetic response of Alaskan tundra plants to increased season length and soil warming. Arct. Antarct. Alp. Res. 40:181-191.

Regeneration process of Antarctic moss Ceratodon purpureus under different PAR conditions

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Mosses are dominant species of terrestrial vegetation and are thought to mainly reproduce asexually in Antarctica. Regeneration is one means of the vegetative reproductions, where gametophyte fragments de-differentiate secondary protonemata, and leafy gametophytes are formed on the protonemata. However, how different intensities of photosynthetically active radiation (PAR) affect the morphogenesis via regeneration remains unclear.

Gametophytes of the Antarctic dominant species Ceratodon purpureus collected in the ice-free area around the Syowa Station were cultured in the laboratory, and tip of the proliferated gametophytes (gametophyte fragments) were subsequently cultured on agar plates for 28 days under 16h light 8h dark, 20 °C, and four different PAR groups (group 1: ca. 27 µmol m⁻² s⁻¹, group 2: ca.56 μ mol m⁻² s⁻¹, group 3: ca. 154 μ mol m⁻² s⁻¹, group 4: ca. 288 μ mol m⁻² s⁻¹). The number of samples in each group is 8, 10, 9, and 8 respectively. We took pictures of each sample about every 7 days and checked whether secondary protonemata were formed or not, and where leafy gametophytes were formed. Then, we calculated the area of the oval covering the secondary protonemata in the image processing package Fiji. We also calculated the expansion speed of the ovals and the amount of captured light (the area of oval \times PAR intensity).

Some gametophyte fragments under only the highest PAR group (group 4) did not de-differentiate secondary protonemata. The area and expansion speed of the oval were the highest under the lowest PAR group (group 1; Fig. 1). The calculated amount of captured light was high when the expansion area of secondary protonemata was large under lower PAR conditions. New gametophytes were formed from axillary bud (base of the leaf) or on secondary protonemata. Identifying the formation site of gametophytes of some gametophyte fragments in group 3, 4 was difficult due to the cluster of protonemata. Thus, we considered them as "unidentifiable". Gametophyte fragments under the lowest PAR conditions (group 1) formed leafy gametophytes on secondary protonemata (Fig. 2). These results suggest that PAR intensity is important in morphogenesis and growth of secondary protonemata, and that the expansion of secondary protonemata seems to be the way to utilize the limited PAR. In Antarctica, regeneration of C. purpureus may occur under low PAR intensity conditions such as below rock and sand.

Only Axillary bud $\begin{array}{c} \text{Axillary bud} + \text{unidentifiable} \\ n{=}8 \qquad n{=}8 \qquad n{=}5 \end{array}$ Axillary bud + on secondary protonemata n=5 1.0 The ratio of the formation site 0.75 0.50 0.25 0 BRONDS proupl \$roup3 Blonby

Only on secondary protonemata

Fig. 1 Growth of secondary protonemata under various PAR intensity. Error bars show standard deviation.

Fig. 2 The formation sites of leafy gametophytes.

Evaluation of degradation potential of heterocyclic compounds by immobilized Antarctic bacteria

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Heterocyclic compounds have been reported to cause biological mutagenicity, nausea, damage to the central nervous system, and serious adverse effects on organs. Biological methods are gaining attention as a means of cleaning up persistent toxic contaminants in the environment. The objective of bioaugmentation, one of the biological methods, is to achieve high environmental cleanup capacity by introducing specific microorganisms into bioreactors. When incorporating microorganisms into bioreactors, it has been reported that immobilizing microorganisms on beads improves degradation efficiency. In addition to an increase in cell density, immobilization of microorganisms is expected to improve the tolerance of biocatalysts because they can be physically distanced from metabolites and substrates.

In this study, for the purpose of environmental remediation using bioreactors in Antarctica, we evaluated the ability of an indigenous Antarctic bacterium, *Sphingobium* sp. strain BS19, immobilized on beads, to degrade carbazole (CAR), a heterocyclic compound. Strain BS19 was isolated from the coastal soil of King George Island and has been found to possess a CAR-degrading genes for metabolism of carbazole. Cells were immobilized in sodium alginate and calcium chloride beads, dropped into flasks or test tubes containing minimal salt medium containing CAR, cultured by shaking, and the residual CAR concentration was determined by GC-FID. To determine the biomass of beads, CFU/mL was analyzed using the resazurin assay.

In the evaluation of decomposition capacity, the biomass concentration, alginate concentration, and CAR concentration were varied. The biomass concentration was varied to 2%, 5%, and 10% to measure changes in decomposition capacity. Alginate concentrations were varied at 1%, 3%, and 6% to measure changes in decomposition capacity; CAR concentrations of 1% (w/v) and 10% (w/v) were used to measure toxicity resistance. In the long-term degradation test, additional drops of MilliQ and CAR were added to the same flask when CAR was completely degraded, and changes in CAR degradation capacity were measured by returning the medium composition to its initial state until degradation decreased.

In the evaluation of decomposition capacity, the concentration of fixed biomass was varied, resulting in higher biomass in the beads with higher biomass concentration, but no significant difference in decomposition capacity was observed. This suggests that when the biomass of the beads is too high, the density of bacteria in the beads increases, and the substrate is not sufficiently distributed. Alginate concentration change showed that the lower the alginate concentration, the higher CAR degradation capacity. This suggests that if the alginate concentration is too high, the density of material in the beads increases, and the substrate may not be sufficiently available. However, if the alginate concentration is too low, the material strength may decrease, so $2\sim3\%$ is considered optimal. Toxicity resistance tests of beads with varying CAR concentrations showed that at a CAR concentration of 1% (w/v), both beads and free cell could be degraded without problems, but at 10% (w/v), there was a significant decrease in both degradation capacity and biomass. From this, a significant increase in substrate concentration could not demonstrate the toxic resistance performance of the fixed strain.

The results of long-term degradation test showed that the decomposition efficiency increased from the 2nd cycle, and that a high level of decomposition continued for 4 cycles. After that, however, the degradation capacity began to rapidly decline. The measurement of biomass by resazurin assay also showed that the concentration of biomass remained high until the 4th cycle, but the concentration was low after the 5th cycle. In this study, long-term degradation was performed in the same medium. The results showed that the high bead biomass maintained a high degradation capacity. However, in the long-term degradation test in the same medium, the bacteria's own metabolites increased the toxicity of the medium, which ultimately reduced the degradation efficiency.

In the future, it will analyze the degradation capacity and the biomass of the beads when they are transferred to a different medium.

References

Bai, Y., Wang, L., Tao, Q., Lu, S., Zhang, A., Li, K., & Hu, N. Application, performance and mechanism of high-entropy catalysts for degradation of heterocyclic compounds. *Journal of Water Process Engineering*, *56*, 104392, 2023. Rohman, A., & Dijkstra, B. W. Application of microbial 3-ketosteroid Δ 1-dehydrogenases in biotechnology. *Biotechnology Advances*, *49*, 107751, 2021.

Succession and Topography: Effects of the two gradients associated with moraines on soil fungal communities

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Primary succession and topography result in microenvironmental changes and are important processes influencing the community assembly of soil fungi in the Arctic region. In glacier forefields containing a series of moraine ridges, both processes contribute synchronously to fungal spatial diversity. To reveal the synergistic effects of succession and microtopography, we investigated the fungal community structure and environmental variables in the moraines of the Arklio Glacier, Ellesmere Island (Fig. 1). The study site were established at four locations from the top to the bottom of the ridge slope within each of the three moraine ridges of different post-glacial ages (Fig. 1). The location-dependent community composition was equally diverse in both the initial and later stages of succession, suggesting that successional time could alter the effects of microtopography on the fungal community. Moreover, our results suggest that fungal communities at different locations follow different successional trajectories, even if they have passed through the same time lapse. Such a synergistic effect of succession and microtopography of moraines does not allow for parallel changes in fungal communities among moraines or among locations, suggesting that the moraine series contributes substantially to fungal spatial diversity in the glacier forefield.

Figure 1. Map of study location. Location of study site in Ellesmere Island (A), map of the three moraines assessed in this study (B), and sampling designs and each moraine's view (C).

Figure 2. (A) Principal component analysis (PCA) of soil environmental factors. (B) Ordination plot of fungal communities based on OTU data. Principal coordinate analysis was performed using the Jaccard dissimilarity index and the results for principal coordinates 1 and 2 are shown. The color and shape of the points refer to moraine and location, respectively.

Exploring novel microorganisms from lakes in the coastal ice-free area of Lützow-Holm Bay, East Antarctica

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It is estimated that there are millions to about one trillion species of prokaryotes on Earth, but most of them are uncultured, and their detailed properties are unknown (Locey and Lennon, 2016; Amann and Rosselló-Móra, 2016). Isolation and cultivation of these novel microorganisms and elucidation of their physiological ecology is one of the most fundamental and essential topics in microbial taxonomy and ecology (Muragkar et al., 2020). In our laboratory, we isolate and characterize novel microorganisms from various extreme environments. Antarctica is an extreme environment of low temperature, aridity, and oligotrophic conditions (Hirose et al., 2020), and there are lakes and marshes of various sizes in coastal ice-free areas where ice melts in summer, and the surface of the water appears (Laybourn-Parry and Pearce, 2016). These lakes and marshes vary in location, depth, salinity, nutrient concentration, etc., depending on their origin and surrounding environment, and are said to have the most incredible diversity on earth (Laybourn-Parry and Pearce, 2016). Recent studies have revealed that these Antarctic lakes contain various microorganisms, including new species (Kurosawa et al., 2010; Chaya et al., 2019). However, the only lake where the microbial community structure has been studied in detail using next-generation sequencing in the Lützow-Holm Bay coastal ice-free area is Lake Zakuro, and there is no knowledge of microbial diversity or the presence of novel microorganisms in other lakes. Based on the above background, the objective of this study was to comprehensively analyze the microbial community structure in several lakes in the coastal ice-free area of Lützow-Holm Bay, East Antarctica, and to search for new microorganisms.

Eight lake water samples, including bottom sediment, collected between December 26, 2012, and January 22, 2013, in the Lützow-Holm Bay coastal exposed rock area were used in the experiment. Environmental DNA was extracted from each sample using the DNeasy PowerSoil Pro Kit. The 16S and 18S rRNA genes were amplified by PCR using this DNA as a template, and the PCR products were purified and mixed in equal amounts to produce an amplicon library. The amplicon library was prepared by purifying and mixing equal amounts of PCR products and subjected to next-generation sequence analysis. Based on the sequence data, the microbial community structure was analyzed using the QIIME2 pipeline. Molecular phylogenetic analysis was performed for ASVs (Amplicon Sequence Variants) that were presumed to be derived from novel microorganisms.

Psudomonadota, Cyanobacteriota, and *Bacteroidota* were detected in relatively high proportions in all environmental samples, with *Psudomonadota* being detected more frequently in samples from salt lakes than freshwater lakes. Conversely, *Cyanobacteriota* was more frequently detected in freshwater samples than in salt lakes. As a novel group of microorganisms, a total of 81 ASVs of "Patescibacteria," a candidate phylum of bacteria, were detected. Although there have been few successful cultures of microorganisms belonging to this group, and their physiological properties are largely unknown, genome analyses suggest that they have incomplete pathways for amino acid and nucleotide synthesis and are presumed to be symbiotic or parasitic (Brown et al., 2015). Isolation and culture of "Patescibacteria" would provide novel taxonomic insights. Molecular phylogenetic analysis revealed that of the 81 ASVs that were presumed to be "Patescibacteria," 37 ASVs were "Saccharibacteria," 18 ASVs were "Adlerbacteria," 10 ASVs were "Adlerbacteria," 12 ASVs were "Nomurabacteria," 9 ASVs were "Gracilibacteria and 5 ASVs were classified as "Campbellbacteria" (all of them in the candidate category). "Adlerbacteria" was detected in a relatively high percentage of the pomegranate pond samples (about 4.5% of the total), suggesting that the bacteria detected in this study belong to this candidate phylum and are microorganisms adapted to low temperature and high salt content.

In the future, further molecular phylogenetic analysis will be conducted for novel microorganisms other than the candidate phylum "Patescibacteria" detected in this study, as well as for novel eukaryotes. In addition, we plan to perform statistical analysis using R to compare the microbial community structure among samples.

References

Amann, et al. mBio, 7, 10-1128, 2016. Brown, et al. Nature. 523, 208-211, 2015. Chaya, et al. Diversity. 11, 105, 2019. Hirose, et al. Microorganisms, 8, 497, 2020. Kurosawa, et al. Polar Science, 4, 421-429, 2010. Laybourn-Parry, et al. Polar Biology, 39, 2207-2225, 2016. Locey, et al. PNAS, 113, 5970-5975, 2016. Muragkar, et al. Journal of Oral Microbiology, 12:1, 2020.

Fungal succession and metabolic traits on subalpine forest floor moss Hylocomium splendens

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Little is known in cold regions about the relationship between succession and metabolic capabilities of fungi associated with moss profiles consisting of live, senescent, and dead moss tissues in different stages of decomposition (Nagata et al. 2023). The purpose of the present study was to examine the successional changes of fungi in decomposing moss tissues and the relationship between the succession and their metabolic traits as evaluated the pattern of caron source utilization. Samples of live and dead tissues of Hylocomium splendens were collected in a subalpine coniferous forest stand near Shirakomaike Pond (2115 m a.s.l.), Sakuho, Nagano in central Japan. Moss shoots exhibited sympodial branching in which the order of modules (i.e. segment of moss shoot) was easily distinguishable from the live green part of moss on the tip (Segment 1) to the senescent, dead, and decomposed parts near the base (Segment 2 to 4) (Uchida et al., 2001). Fungi were isolated from these moss segments with a washing method, and the ribosomal DNA Internal Transcribed Spacer (ITS) sequences of fungal isolates were analyzed for the determination of operational taxonomic units (OTUs) according to the similarity at the 97% criterion and for the taxonomic assignment (Hatano et al. 2022). The successional trends of individual OTUs were quantified by the succession index (M) according to the formula of Hasegawa (1997). Fungal isolates were then tested for their carbon source utilization using Biolog EcoPlateTM, with 31 different carbon sources for the assessment of their metabolic traits. A total of 244 fungal isolates were obtained from 120 moss segments and were grouped into 44 OTUs, including 35 OTUs in Ascomycota, 8 OTUs in Mucoromycota, and 1 OTU in Basidiomycota. Of the 44 OTUs, 25 OTUs (57%) occurred only at one sample and were regarded as singletons, and the other 19 OTUs (43%) were non-singletons. The most OTU-rich genus was Penicillium (10 OTUS), followed by Trichoderma (8 OTUS) and Cordyceps, Pestalotiopsis, Mortierella, Mucor, and Podia (each 2 OTUS). The succession index of 44 OTUs ranged from 1.0 to 4.0, and that of 19 non-singleton OTUs ranged from 1.7 to 3.5. The mean value of succession index was not significantly different between Ascomycota and Mucoromycota for the 19 non-singleton OTUs. The 44 OTUs exhibited potentials to utilize an array of 31 substrates in Biolog EcoplateTM, with the greatest mean metabolic activity found for Tween 80 and Tween 40 and the lowest mean metabolic activity for glucose-1-phosphate and α ketobutyric acid. Principal component analysis discriminated the 44 OTUs according to the patterns of carbon source utilization. The first two principal components (PC1 and PC2) together accounted for 52% and 12% of the total variance, respectively. PC1 was positively associated with Tween 80, D-mannitol, and L-asparagine and was not significantly different between Ascomycota and Mucoromycota, whereas PC2 was positively associated with D-mannitol and negatively with Tween 40 and was significantly greater in Ascomycota than in Mucoromycota. The succession index of 19 non-singleton OTUs were significantly and positively correlated with α -ketobutyric acid and p-malic acid (P < 0.05). The model selection of the generalized linear models by Akaike's Information Criterion concluded that the model with six carbon sources (α -ketobutyric acid, L-serine, 4-hydroxy benzoic acid, D-malic acid, D-galacturonic acid, and L-phenylalanine) was the best model accounting for the succession index of 19 non-singleton OTUs. The best model showed that the succession index of fungi was significantly and positively related with the activity to metabolize α -ketobutyric acid, 4-hydroxy benzoic acid, and D-malic acid and significantly and negatively with L-serine, D-galacturonic acid, and L-phenylalanine. These results demonstrated that the metabolic traits of fungi were closely associated with their successional patterns in decomposing moss tissues on the subalpine forest floor.

References

- Hasegawa, M., 1997. Changes in Collembola and Cryptostigmata communities during the decomposition of pine needles. Pedobiologia 41, 225-241.
- Hatano, Y., Yoshida, T., Matsuzuka, S., Osono, T., Hobara, S., Hirose, D., Tanabe, Y., Kudoh, S., Uchida, M., 2022. Occurrence, hyphal growth rate, and carbon source utilization of fungal isolates from continental Antarctica. Polar Sci. 31, 100738.
- Nagata, Y., Osono, T., Hasegawa, M., Hobara, S., Hirose, D., Tanabe, Y., Kudoh, S., Uchida M., 2023. Application of the Biolog EcoPlateTM technique for assessing the microbial metabolic diversity in moss profiles of continental Antarctica. Polar Sci. 35, 100924.
- Uchida, M, Nakatsubo, T., Tanaka, K., Nakane, K., Horikoshi, T., 2001. Decomposition and ergosterol content of the moss *Hylocomium splendens* litter under various climatic conditions. Polar Biosci. 14, 71-78.

Effect of gelatin on PLA Degradation in Massilia sp. N-3 isolated from Antarctic soil

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Plastics are essential materials in our daily lives. Consequently, their production has been increasing annually, raising concerns about environmental pollution. In response, there has been growing interest in bioplastics. Among these, polylactic acid (PLA), which is made from crops such as corn, is anticipated as a promising alternative to conventional petroleum-based plastics due to its excellent material properties.

PLA is a biodegradable plastic that microorganisms can degrade into water and carbon dioxide. However, it has been reported to be difficult to degrade in the natural environment and is often considered virtually non-biodegradable under common environmental conditions.

Since the first report of PLA-degrading actinomycete *Amycolatopsis* sp. HT30, many researchers have shown interest in the degradation of PLA by microorganisms. However, many of the PLA-degrading bacteria reported to date have been actinomycetes. Therefore, further research into PLA degradation across a more diverse range of species is required.

Massilia sp. strain N-3, a microorganism isolated from Antarctic soil can degrade PLA, and its whole genome sequence has been elucidated. It has also been observed that the degradation of PLA is enhanced with the addition of gelatin, although the mechanism remains unknown. The objective of this study is to investigate the mechanism by which gelatin addition promotes PLA degradation in strain N-3.

It is widely reported that PLA is predominantly degraded by hydrolytic enzymes, such as proteases and lipases (Penkhrue, 2015). Accordingly, in this experiment, the production of hydrolytic enzymes in the presence of added gelatin was initially investigated. Measurement of hydrolytic enzyme activity was performed using substrates such as *p*-nitrophenyl butyrate (pNPB), *p*-nitrophenyl octanoate (pNPO), and *p*-nitrophenyl laurate (pNPL). Strain N-3 was cultured in a minimal salts medium (MSM) supplemented with various carbon sources: PLA, glucose, gelatin, and PLA with gelatin. Samples were collected at regular intervals to measure protein concentration, viable cell count, and enzyme activity. The experimental results indicated that the addition of gelatin significantly increased the production of hydrolytic enzymes.

Subsequently, differences in the expression of genes between the media containing gelatin as the sole carbon source and those containing both gelatin and PLA were examined. Total RNA was extracted from strain N-3 cultured in each medium, and cDNA was synthesized via reverse transcription. Using this cDNA as a template, PCR was conducted with primers targeting predicted degradation-related genes. Gel electrophoresis results showed no differences in the expression of the predicted genes. From this study, it was found that the addition of gelatin enhances the production of hydrolytic enzymes in strain N-3. Additionally, the lack of differential expression in the predicted genes for PLA degradation suggests that the enzymes responsible for metabolizing gelatin might also contribute to PLA degradation.

Curently, this study has not provided information on differences in the amount of expression or the expression of genes other than those predicted. Therefore, future studies will involve validating gene expression via quantitative PCR and conducting comprehensive analyses of expressed genes using RNA sequencing.

References

Penkhrue, W., Khanongnuch, C., Masaki, K., Pathom-Aree, W., Punyodom, W., and Lumyong, S. Isolation and screening of biopolymer-degrading microorganisms from northern Thailand. World Journal of Microbiology and Biotechnology, 31, 1431, 2015.

Prey pursuit strategy of the Humboldt penguin, determined by animal-borne data loggers

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Penguins are interesting animals that, despite being closely related to the order Procellariiformes, have adapted to an underwater environment and lost the ability to fly, which they once possessed. As top predators in marine ecosystems, they play a crucial role in regulating the food web. Accordingly, numerous studies have been conducted on their feeding ecology and behavior; however, few have explored the geometric routes they take to track their prey (i.e., pursuit strategies).

Pursuit strategies used by predatory animals are often categorized into three strategies: pure pursuit, deviated pursuit, and parallel navigation. Pure pursuit (Figure 1) involves a predator tracking its prey by continuously adjusting its direction to keep the prey directly in front. Deviated pursuit (Figure 2) involves the predator aiming at a consistent angle ahead of the prey's current direction of movement. Parallel navigation (Figure 3) involves the predator maintaining a fixed absolute bearing relative to the prey's movement in a global coordinate system. Although the number of studies is limited, it has been reported that many birds, particularly those that hunt in open environments, use parallel navigation, while many fish are known to use pure pursuit or deviated pursuit strategies. Therefore, understanding the pursuit strategies of penguins, which are birds that hunt underwater, and comparing them with those of other birds and fish, will help determine whether the differences in environment (water vs. air) or the phylogenetic background influence the pursuit strategies employed by animals.

In general, pursuit strategies of animals have been identified by video recording the coordinates of predator and prey or by attaching GPS data loggers to them. However, these methods are not applicable to penguins, as they swim underwater where GPS data is not usable and their movement range is too large for effective video recording. To address these challenges, we equipped the Humboldt penguins (*Spheniscus humboldti*) with specialized data loggers, each incorporating a wide-angle camera, an accelerometer, and a gyroscope. Data were collected from 7 penguins during two experimental trials, in which 500 or 1,000 live Japanese anchovies were released into a tank. We successfully recorded 70 instances of pursuit behavior. Using footage from the wide-angle cameras, we estimated the position of the prey relative to the penguins' heading direction, while the accelerometer and gyroscope data allowed us to estimate the three-dimensional postures of the penguins. Our analysis revealed that Humboldt penguins employ a strategy consistent with pure pursuit, where they consistently keep the prey directly in front of them while pursuing.

Figure1. Pure pursuit

Figure 2. Deviated pursuit

Figure 3. Parallel navigation