南極とカムチャッカ氷河における微量元素と耐性遺伝子の関連性

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要旨

南極・ドームふじとカムチヤッカ半島・ウシュコフスキー氷冠にて採材されたアイスコアサンプルを使用し微量元素組成、生物相の検証を年代情報と関連して行った。サンプル溶解に際してはアイスコア外壁に付着物からの汚染を防ぐため、アイスコアをメタノール、純水でゆっくりと融解し、氷の中心部に存在する成分を使用して分析を試みた。これら液体中の微量元素濃度を粒子励起X線分析法（PIXE）により測定した。また、溶解サンプル中の生物由来成分を遠心分離にて沈降させ、それらから核酸を抽出し、増幅キットと PCR にて 16S-rRNA と、水銀、銅、カドミウムに耐性も持つ遺伝子の検出を試みた。PIXE 分析により 28 の元素が検出され、その中でも、カリウム、塩素、カルシウム、マグネシウム、ケイ素、水銀成分は両サンプルにて認められ、カムチャッカ半島のサンプルからはイットリウムが検出された。水銀も両地点のサンプルから検出され、深度によっての濃度差が認められた。すべてのアイスコアのサンプルで16S-rRNAが確認されバクテリアの存在が示唆されたが金属耐性の遺伝子は検出されなかった。これらの結果から、アイスコアには海洋由来と考えられる成分が存在し、還移元素の存在では地域差が認められた。またバクテリアがすべてのサンプルにて検出されたことから、これらの成分は雲凝結核などの降雪や大気由来降下物が大きく関与していることが考えられ、さらな詳細な微生物相の解析は過去の状況を知る上で貴重な情報を提供してくれる可能性が示唆された。

Comparison of trace elements and resistant genes in Antarctic and Kamchatka ice cores

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Abstract

Spatially separated ice core samples from Dome Fuji and Ushkovsky glacier were selected as remote and relatively near human settlements, respectively. Only a center part of ice core samples were melted carefully to avoid the contaminants during the transportation, handling, and storage. The concentrations of trace elements in the liquid samples were analyzed with a Particle Induced X-Ray Emission (PIXE). The melted samples were centrifuged for DNA recovery and extraction by Instagene™ matrix kit, then further amplified with whole genomic amplification kit for PCR with 16S-rRNA and mercury, copper, and cadmium resistant genes. Among 28 elements detected with PIXE, relatively high concentration of potassium, chlorine, calcium, magnesium, and silica were measured in all samples; yttrium was found only in Kamchatka ice samples. The results from a mercury analyzer indicated Dome Fuji ice core samples showed a decreasing trend with greater depth of ice cores over the period of ca. 1350 to 2800 BP; there was no clear trend with Kamchatka ice samples from ca. 1950 to 1980. The results from the PCR with 16S-rRNA analysis indicated presence of bacteria in all sample. There were no metal resistance genes detected in all ice core

samples examined, despite the different level of mercury and other metal concentrations. This investigation confirms the relevance and importance of atmospheric depositions of bacteria in the ices. Further investigation with meta-genomic approach to measure diversity of microorganism flora may help to increase the understanding of different spatial and temporal variations of polar environment through the ice cores as information sources of paleoscience.

1. Introduction

Antarctica and Kamchatka are located on or near the polar region, the extreme low temperature condition makes less dense biological system and preserves the past conditions with less human disturbances. Since the lower biological diversities and fewer numbers of chemical substances are expected in ice samples from such regions, less complex chemical and biological systems are expected. The ice core samples with separate isotope ration analysis provide temporal information, which allow us to consider the past environmental conditions together with time information. Biological and chemical information together with temporal information may provide further knowledge in a field of paleoscience. Previous investigations with drift ice samples form Antarctica and Okhotsk regions indicated presence of mercury and mercury resistant genes, *merA*. Therefore, further investigation of heavy metals and corresponding resistant genes in different ice core samples may increase the understanding of the relationship in different environmental conditions. In addition to the measurement of a paleobiological aspect, less diversified condition may provide as a suitable baseline model to understand the interaction between certain chemical substances and microbial flora. This study focuses on the analyses of metal and metal resistant genes in ice samples from inland part of the arctic regions where snow precipitation contributes mostly to form the ice cores.

2. Material and method

Table 1. Ice core samples from Antarctica and Kamchatka glacier.

Location	Sample	Depth (m) From surface	Estimated age of ice
Dome Fuji, Antarctica 77°19' S 39°42' E	DF-63	63.57	1405 BP
	DF-85	85.34	2100 BP
	DF-107	107.86	2850 BP
Ushkovsky, Kamchatka 56°04' N 160°28' E	KM-70	70.79	1980
	KM-110	110.50	1970
	KM-147	147.59	1950

Total of 6 ice samples from Antarctica and Kamchatka ice cap were examined for concentrations of trace elements, existent of bacteria, and metal resistant genes. The age of ice cores were roughly estimated from several reference point obtained from isotope analysis provided by institute of low temperature science, Hokkaido University. The ice core samples were obtained

from Dome Fuji, located inland part of Antarctica and another set of samples from Ushkovsky ice cap in Kamchatka, (+ 3903 m a.s.l.). Table 1. Indicates sample information about the depth of the examined ice core from the surface and estimated age of ice cores samples. These ice core samples were stored at below -30℃ until the samples were melted and analyzed.

Prior to the series of the analyses, the ice samples were carefully rinsed with 99% ethyl alcohol and then washed with deionized distilled water for three times to remove contaminants from surface of the ice, only the core part of the ice samples were melted and examined further. To minimize the risk of contamination from ambient air, the melting process of ice samples was conducted inside the clean bench. First, the melted sample was filtered with 0.8 μm pore size membrane filter to remove coarse particles; sequentially 0.22 μm pore size membrane filter was deployed to collect remaining particles. The remaining liquid samples from the first filtration step were further analyzed with Particle Induced X-Ray Emission (PIXE) to determine wide range of total elements. The liquid samples with second filtration step with 0.22 μm pore size membrane filters were processed with Instagene™ matrix kit and EXTRAGEN MB kit to extract DNA. To verify existence of bacteria, a PCR test using primers for 16s-rRNA gene was performed. For further analysis, Whole Genomic Amplification (WGA) process was deployed to amplify the whole genomic information by PCR, and specific metal resistant genes were examined as follows: Cu with *tcrB*, Cd with *cadA*, Hg with *merA*, and methyl mercury with *merB*. After the melting process, all the samples were kept at 4 °C until the analyses were conducted.

3. Results

Table 2. PIXE analysis result with mean concentrations (S.D. 1σ) of selected elements (μg/L).

Sample	Si	P	S	Cl	Mn	Cu	Rb	Sr	Y	Nb	Hg
DF63	485 (220)	- *	N.D. `	955 (190)	0.6 (-)	1.0 (0.3)	0.1 (-)	-	N.D	0.1 (-)	N.D.
DF85	841 (250)	99.9 (88)	167 (260)	869 (210)	0.5 (-)	1.7 (1.3)	0.5 (0.3)	2.5 (1.4)	N.D	1.2 (0.11)	1.0 (-)
DF107	877 790	318 (110)	175 (120)	627 (400)	0.4	1.2 (0.8)	4.2 (2.3)	1.0 (0.8)	N.D	N.D.	0.3 (-)
KM70	685 (74)	N.D.	109 (37)	473 (110)	N.D	1.4 (0.6)	3.7 (3)	2.2 (0.8)	1.2 (-)	1.8 (-)	2.4 (-)
KM110	326	17.5 (9)	1230 (72)	612 (170)	11.1 (3)	7.6 (2)	4.5 (3)	3.7 (2.7)	0.8 (-)	2.5 (-)	0.3 (-)
KM147	391 (150)	1.1 (-)	134 (64)	331 (78)	6 (-)	0.9 (0.2)	6.1 (-)	4.5 (-)	0.9 (0.7)	1.2 (-)	3.9 (-)

*- : Not Available, ` N.D.: Not Detected

a) PIXE

Result from the PIXE analysis shown in Table 2, which only indicates selected 11 elements out of 28 detected elements. Unlisted 17 elements, due to the relatively low concentrations, are Mg, Al, K, Ca, Ti, V, Cr, Fe, Co, Ni, Zn, Ga, As, Se, Br, Mo, and Pb. Among all the elements, relatively high concentrations of Mg, K, Ca, Cl, and S were found in all samples. DF63 had no detection of P nor S.

Manganese was found highest with 11.1 (± 3) μg/L in KM110, but 0.3-0.6μg/L with DF samples. Yttrium was only found in Kamchatka ice samples with 0.8-1.2 μg/L. Mercury was detected in all ice samples except DF63 with PIXE measurement. For the mercury data, Kamchatka KM 147 showed the highest value of 3.9 μg/L and the DF 63 has lowest value of 0.6 (± 0.2) μg/L. Kamchatka ice samples tend to have higher value than Dome Fuji samples.

b) Genetic analysis

PCR of 16S-rRNA genes with Instagene™ and EXTRAGEN extraction kits with the second filtered materials indicated presence of bacteria in all ice samples. However, the respective resistant genes of Cu, Cd, and Hg, the *tcrB, cadA, merA,* and *merB,* were not detected from all the examined samples.

4. Discussion

The result indicated that there were larger variation of Hg level with DF samples, this may be caused by the different time scale of samples. The DF sample age was an order of 1000 years; however, KM sample age was an order of 30-40 years, these differences may be reflected with greater environmental and climate change over the longer time period. This may be a part of reason for DF samples to have wider variation of Hg concentrations. PIXE measurement indicated relatively high concentrations of K, Cl, Ca, Mg and Si in all examined samples. Presence of such alkali elements may be associated with deposition of marine origin aerosols. Since the locations of the sampling sites are surrounded by ocean and most of the land surfaces are covered with less interactive ice and/or snow, marine aerosols may travel great distance without much of modification or interaction with the surface materials. Yttrium was measured only in Kamchatka ice samples; this might be associated with long distance transport from Asian continent where the rare earth metals were found. This indicates possible atmospheric deposition via long distance transport as one of possible modes; however, use of such elements as a tracer to locate their emission source is difficult task to achieve. There is an effort to classify spatial distributions of desert sand in China-Mongolia region with Sr and Nd isotope ratios as fingerprints (Nakano et al., 2004). With the currently available isotope measurement with mass spectrometric technique to determine Sr isotope ratios, still it requires few hundred mg of sample with an order of 10 ppm range concentration. The results from deposited Sr in the ice core samples indicate less than 10 ppb levels; it would require a large amount of ice samples and pre-concentration steps to implement such method. Therefore, this method to deploy the trace element compositions as a fingerprint to identify source origin may have its limitation.

From the result of 16S-rRNA analysis indicated the presence of bacterial gene in all the ice samples examined. Since the investigated ice samples were mostly composed of accumulation of snow precipitation, the result with presence of bacteria accords with the observation of abundant bacteria found in snow precipitations by Christner et al. (2008). Despite the presence of bacteria and certain level of copper and mercury, the examined ice core samples did not indicate copper or mercury resistant genes. Although, previous investigation with analysis of ice samples showed about equal amount of mercury levels and the *merA* was detected. From these results the *merA* gene as a function of defend against Hg was not selected within snow or transition period from snow to ice, but it probably had such characteristic from the initial stage as snow crystal and/or deposit with some form of dust. Thus the variations of Hg concentration do not have a direct effect to act as a selection pressure to the microorganism flora of each ice samples. Similar investigation of the

merA and total mercury concentration in different depth of snow samples from Greenland indicated a presence of *merA* in snow with relatively low concentration of total Hg in that site; there was no positive correlation, thus bacteria were not influenced solely by the presence of Hg to express their resistant characteristics (Moller, 2011). Moller et al. and this study conclude ice core samples possess unknown mechanism within microorganism flora that affects the presence of the *merA* gene.

The variability of microorganism flora in ice may reflect with what had deposited via atmospheric transport. We suspect to have certain microbes or their fragments as CCN, which act as key components of deposits. Further investigating of microorganism flora or whole genomic diversity in ice with temporal information may help to characterize the past atmospheric condition, which may provide the useful information in the field of paleo-science.

Ice nucleators, the core part of the snow crystals, have been reported to they can be bacteria and other biological components; therefore, the snow might have brought some foreign microbes into the ice microbial flora. In addition to the trace element analyses, further understanding of specific gene information of microbes and microorganism flora may bring a new insight for the field of paleo-science and other environmental sciences.

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