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Short Communication

Application of real-time PCR array to the multiple detection of antibiotic resistant genes in glacier ice samples

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Glacier environments harbor cold-tolerant bacteria (Amato et al., 2007; Christner et al., 2003; Segawa et al., 2005). However, a variety of bacteria such as soil and enteric bacteria have been detected from these highly oligotrophic and psychrophilic environments (Segawa et al., 2005; Sheridan et al., 2003). The origin of these bacteria remains unknown. These bacteria may have been supplied at least in part from animals by means such as bird droppings (Sjölund et al., 2008). There are other plausible transmissions, such as local and global atmospheric circulation. In the case of the global atmospheric circulation, a glacier environment may receive bacteria originating from human industrial activities, although most glaciers are located at a distance from such sites of human activities.

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Emergence of antibiotic resistant bacteria has been considered as a human impact on the environment (Cabello, 2006; Chee-Sanford et al., 2001; Goni-Urriza et al., 2000). The prevalence of antibiotic resistant genes in the glacier environment can be used as an indicator of human impact on the global environment.

In this experiment, the prevalence of antibiotic resistant genes in glacier ice samples taken from various locations in the northern hemisphere (Alaska, Central Asia) and Antarctica were evaluated by real time PCR array approach using a Taqman probe system.

We collected samples of the glacial ice (1 cm in depth) or cryoconite hole samples (Table 1) using a sterilized stainless steel scoop or sterilized syringe, respectively. The samples were stored in 50-ml sterile plastic conical tubes (Iwaki, Tokyo). All samples were kept frozen and transported to the Tokyo Institute of Technology, Japan or National Institute of Polar Research, Japan.

Bacterial DNA was extracted as follows within a Class 100 Clean bench (Sanyo, Tokyo, Model MCV-131BNS) at the National Institute of Polar Research (Tokyo). Ice samples were melted and a portion (1–

Table 1. Glacier sampling site.

Sampling glacier	Area	Lat., long.	Altitude (m)	Sampling date	Reference
Gulkana Glacier	Alaska Range, Alaska	63° 28′ N, 145° 41′ E	1,585	2001 Aug.	Takeuchi et al., 2001
Ürümqi Glacier No. 1	Tien Shan Mountains, China	43° 06′ N, 86° 48′ E	4,090	2006 Aug.	Takeuchi et al., 2008
Rink Crags area	James Ross Island, Antarctica	63° 55′ S, 58° 10′ S	490	2006 Feb.	Fukui et al., 2008

2 ml) of each was centrifuged at 22,000 × *g* for 15 min to obtain pellets. The pellets were then transferred to separate 2.0-ml safe-lock microcentrifuge tubes and subjected to DNA extraction using a FastDNA spin kit for soil and a FastPrep FP120 instrument (Q-BIOgene, Heidelberg, Germany).

A part of DNA was used for PCR amplification, cloning, and sequencing of the 16S rRNA gene sequences. The number of PCR cycles was kept to a minimum to reduce PCR-dependent biases in amplification that could destroy the original composition of the bacterial DNA in the samples and to reduce errors caused by chimera formation resulting from nucleotide mis-incorporation (Polz and Cavanaugh, 1998; Qiu et al., 2001; Wilson and Blitchington, 1996). 16S rDNA was amplified using primer set 27F and 1492R (Lane, 1991) using Prime Star HS DNA polymerase (TaKaRa, Kyoto), used under the following conditions: 3 min of initial denaturation at 94°C; 15 − 20 cycles of 98°C for 10 s, 52°C for 15 s, and 72°C for 120 s; and final extension at 72°C for 7 min. PCR products were then purified using a MinElute PCR Purification Kit (Qiagen, Hilden, Germany) and cloned into a Zero Blunt TOPO PCR Cloning Kit for Sequencing (Invitrogen, Madison, WI, USA). *Escherichia coli* DH5a (TaKaRa) was transformed with the cloning vector, and the transformants were selected by blue-white selection on Luria-Bertani agar plates containing 100 g/ml ampicillin, 1 mg/plate 5-bromo-4-chloro-3-indolyl-beta-D-galactoside (X-Gal), and 2.38 mg/plate isopropyl-beta-D-thiogalactopyranoside (IPTG). Insertion of the appropriate size of DNA was determined by colony PCR amplification with a universal primer set (M13F and M13R) targeting both sides of the cloning vector. Colony PCRs using *Ex Taq* polymerase (TaKaRa) were performed under the following conditions: 3 min of initial denaturation at 94°C followed by 30 cycles (94°C for 30 s, 55°C for 30 s, and 72°C for 120 s) and a final extension at 72°C for 7 min. Sequencing of the inserted DNA fragments was achieved using Big Dye Terminator v3.1 and an ABI automatic sequence analyzer (model 3130xl; Applied Biosystems, Tokyo) with the universal primers M13F and M13R. The amplified sequences were checked for chimeras using Ribosomal Database Project II Chimera Check and the Bellerophon Server. Sequences were clustered with CD-HIT software at 97% sequence identities (Li et al., 2001). Homology searches were performed against the DDBJ/EMBL/GenBank nucleotide sequence database using the BLAST program.

Extracted DNA was amplified using a RepliG Mini kit (Qiagen) according to the manufacturer's instructions for the real-time PCR of antibiotic resistant genes. Amplified products were purified with a Microcon YM-100 column (Millipore, Billerica, MA, USA), then incubated with Phi29 polymerase (Epicenter, Madison, WI, USA) and digested with S1 nuclease (Invitrogen) described in Zhang et al. (2006) for real-time PCR template. Final concentrations of DNA were ca. 450 ng/μl. LightCycler®480 Real-Time PCR System (Roche Applied Science, Tokyo) for qPCR was used to amplify 93 selected antibiotic resistant genes and partial 16S rRNA genes as a positive control (Table 2). The present detection system for antibiotic genes was based on the 96-well format real-time PCR. Therefore, the number of tested gene sequences in one assay was limited to 96 for practical reasons. The selection was made from the comprehensive reviews of the literature (Aarestrup, 2006; White et al., 2005) and a microarray study (Perreten et al., 2005). Primer-sites and probe-sites were selected with the aid of Universal Probe Library Assay Design Center of Roche Applied Science (http://www.roche-applied-science.com/sis/rtpcr/upl/index.jsp?id=UP030000). Prior to this step, complete sequence data were retrieved from databases (DDBJ/EMBL/GenBank); therefore partial sequences in the data base was omitted, and aligned by Clustal W (Thompson et al., 1994). Consensus sequences for particular antibiotic resistant genes were applied to the above on-line program. Selection of primer sites and probe sequences for particular genes was abandoned if there was no hit for primer or probe site from the program. LightCycler®480 Taqman Probe Master® (Roche

Applied Science) was used to amplify the antibiotic resistant genes. The detection and quantification of amplicons was achieved with the aid of a mono color hydrolysis probe system. The reaction mixture (10 μl) contained 1 pmol probe, 2 pmol forward and 2 pmol reverse primer, 5 μl Taqman Probe Master and 3.6 μl of template DNA solution (ca. 9 ng/μl). The temperature cycle was initiated with a denaturation for 5 min at 95°C. The initial denaturation was followed by 50 cycles of 95°C for 10 s and 60°C for 20 s. The record and the analyses on amplification curve was done according to the manufacturer's instructions (Roche Applied Science). Cycle numbers for the crossing point of amplification (CP number) was determined by the Second Derivative Maximum Method. All samples were subjected to triplicate analyses.

In this study, we sequenced 158, 165, and 83 clones of 16S rDNA respectively for the samples from Gulkana Glacier, Ürümqi Glacier and the small ice cap on Rink Crags (406 clones in total). Low-cycle PCR amplification of nearly full length of 16S rDNA revealed that the samples from the three sites contained a total of 79 OTUs (operational taxonomic units). A total of 32, 15, and 39 OTUs were detected in the samples collected at Gulkana Glacier, Ürümqi Glacier and the small ice cap on Rink Crags, respectively. The large clone percentages for the bacteria belonging to the *Beta-proteobacteria* were detected in samples from Gulkana Glacier (54%) and Ürümqi Glacier (79%). But *Alpha-proteobacteria* and *Actinobacteria* were frequently detected in the small ice cap on Rink Crags (26%). Proteobacteria were predominant or present at relatively higher proportions in all the samples. This agrees with several previous studies. For example, Skidmore et al. (2005) reported that *Beta-proteobacteria* were abundant in the clone library in the sediment of the glacier outlet stream from an Alaskan glacier and those in the basal ice samples of a glacier in Ellesmere Island, Canada. Nemergut et al. (2007) also reported that *Beta-proteobacteria* were abundant in the clone library in soil samples of a deglaciated area near a glacier in the Peruvian Andes.

The results of real-time PCR array are shown in Table 2. Among 94 sets of primers and probe, the bacterial 16S rRNA gene was always detected at nearly the same intensity for all the test samples (ca. 25 cycles), although Antarctic ice sample showed a relatively smaller copy number as indicated by the greater CP number (approximately 28). In the negative control sample, the insignificant signal (45) was detected for 16S rRNA genes. Since all the CP numbers for 16S rRNA genes in ice samples were nearly constant, it is suggested that the level of bacterial genomic DNA in the samples was nearly the same in this study.

The genes *cat1* and *tet(C)* were detected with a relatively constant CP number (from 30 to 40) for all the samples including the negative control. These genes should be considered as contaminants in the present experiment, although the sources of contamination are still unclear. If these genes were omitted from consideration, the detected genes were *aacC1*, *aph(6)*, *ampC*, *blaCTX-M*, *cmrA*, *msr*A/B, *tet(D)*, *tet(E)*, and *tet(G)*. These genes were detected in the samples either from Gulkana Glacier or Ürümqi Glacier. None of these genes were detected in the Antarctic ice sample. Emergence and dissemination of antibiotic resistant organisms or their resistance genes has been caused by the human use of antimicrobials (Aarestrup, 2006; Chopra and Roberts, 2001; White et al., 2005). Many studies have documented the presence of antibiotic resistant organisms or their resistance genes in soil and waters adjacent to human activities such as a hospital or an agriculture field (Agerso et al., 2006; Andersen and Sandaa, 1994; Furushita et al., 2003; Heuer et al., 2002; Jacobsen et al., 2007; Koike et al., 2007; Malik et al., 2008; Petersen et al., 2002; Pruden et al., 2006; Sengeløv et al., 2003). At the same time, it is suggested that antibiotic resistance genes can propagate and persist in an antimicrobial-free environment through the clonal expansion of resistant strains and the horizontal transfer/recombination of mobile genetic elements harboring resistance genes (Pallecchi et al., 2007). The detection of antibiotic resistance genes in the glacier environment seems to be a typical case for the antibiotic resistance gene in the antibiotic-free environment. As indicated above, the spread of antibiotic resistance is facilitated by the association of resistance genes with mobile genetic elements. Horizontal gene transfer is a key phenomenon for antibiotic resistance genes to persist in a new environment (Van Elsas and Bailey, 2002). Indeed, the antibiotic resistance genes detected here have been reported to reside on the mobile genetic elements (Agerso et al., 2006; Bauernfeind et al., 1996; Bradford et al., 1997; Chopra and Roberts, 2001; Desomer et al., 1992; Furushita et al., 2003; Matsuoka et al., 1997; Nagy et al., 1997; Van Overbeek et al., 2002; Wohlleben et al., 1989).

The origin of these resistance genes in the glacier

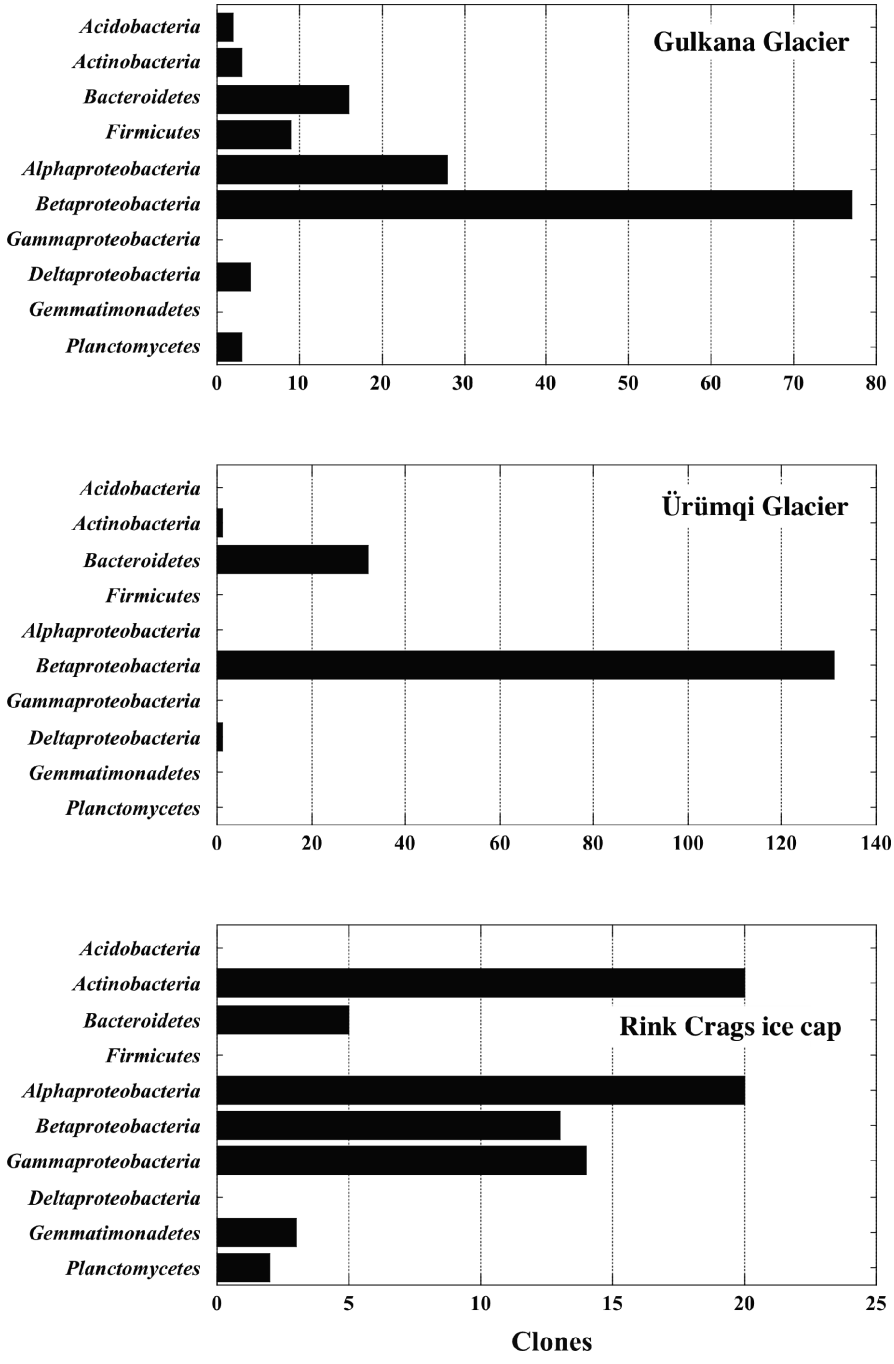

Fig. 1. Distribution of bacterial phyla detected from the ice samples taken in Alaska (Gulkana), Central Asia (Ürümqi) and Antarctica (Rink Crags).
Details, see Table 1.

environment is a vital question to be answered. However, the source-tracking for the antibiotic resistance gene is not an easy task. Some molecular studies tried to answer this question; Koike et al. (2007) presented a successful source-tracking of the *tet(W)* gene in the limited area around swine confinement facilities. These authors applied phylogenetic analyses to the source-tracking survey using the nearly full sequence of this gene. By contrast, the major purpose was to develop the real-time PCR array for environmental antibiotic resistance gene in our study. Therefore, the present results, which were based on the shorter sequences, remained at the level of first screening for antibiotic resistance genes. Further studies about the phylogeny

Table 2. Real-time PCR detection of antibiotic resistant genes from ice samples of glaciers.

Class	Gene	Primers		Probe No.	Reference	Alaska	China	Antarctica	Negative control
		F	R						
Aminoglycoside resistance	*aac(2′)Ib*	gacacagccagaggcatgta	cagtccctcgtcgtcctc	#83	U41471				
	aac(2′)Ic	gcagactgtacgcctcacg	ttggtgccagtaccgatgt	#37	U72714				
	aac(3)	cttttgaccgcggaacag	gctcgaacttgggtagaacg	#149	I016157				
	aac(6′)	ctggttcgagctctggttg	cgtttggatcttggtgacct	#150	EF031554				
	aacC1	tgccttcgaccaagaagc	actacgcgcctgctcaaa	#113	AY139604	44.2±1.87			
	aacC2	gcctcacttaaagcgattggt	tcccatcacagtgccagtc	#63	EU022315				
	aacC4	caacacgacgctgcatct	tgtctcggcaccccatag	#147	DQ241380				
	aacC9	catgctctcggcaggaat	cggcgtcaaacaggtagc	#122	M55427				
	aadA	aaccttaacgctatggaactcg	cgtaagcactacatttcgctca	#26	AY602406				
	aadB	cggggatgagttacttgactg	cctccgcgatttcatacg	#113	DQ388125				
	aadE	tgctattgatcattttaatcagattgt	gatgccgaccttccatgata	#146	AY602212				
	aadK	cccgaattaggtaattggttttc	tcttctgcttcacgaattgga	#145	M26879				
	ant	taagaagggcttgtttgagtcct	gctggatgtacccagaaacg	#14	AY167643				
	ant(4′)	tttctggaaaggcctagtcg	acgggattcgttttgagtga	#44	AJ506108				
	aph(2′)Ib	agtacagatgatttcgggaaaga	ggcgccttatgctgatagtatt	#4	AF207840				
	aph(2″)Id	caaaaatctgccgaagcaat	cttaaaaatcgggccaggtc	#27	AY743255				
	aph(3′)	atgcctgcttgccgaata	ccacagtcgatgaatccaga	#31	EF067857				
	aph(6)	gatccggaggaccgtagc	caggtcccagtgcaggac	#28	AY971801	40.2±3.00	43.3±0.62		
	armA	tggatttcctacagctttttcat	ccatagctggtaattctcttcca	#133	EF158297				
	strA(#1)	ccgtcaatcccgacttctta	cacgagccaaaagatcgag	#153	AF321548				
	strA(#2)	gatccggaggaccgtagc	caggtcccagtgcaggac	#28	AJ862840				
	strW	aggcctacgaagccgaac	tgagctcgaccgtcaggta	#17	X89010				
	strX	ggctccgtctgatagtggaa	ccagtgcaatctctcgttca	#75	X89010				
Beta-lactam resistance	*mecA*	acaatcgcacatacattaatagagaaa	gccataatcatttttcatgttgtta	#140	Y00688				
	ampC(#1)	aaagcaatccacgtttcacc	gttggttttgacaccatacgc	#42	AB016611				
	ampC(#2)	ggctacggggtgaagacc	atgcaggttggcatcgac	#62	AB211125	35.9±0.82	39.6±3.68		
	blaACT	gctggaccatacgtggattaac	ggtatccccaggcgtaatg	#144	EF508682				
	blaADC	gcccttggcttaaaacatagc	aactcgaatcggctgattttc	#115	EF409593				
	blaB	tggttgtgtggttccctaaag	ggtctttcgaatcaccactttt	#75	AF189304				
	blaCFI	ggactgggttacctgcaaaa	ggcgaggtctatcgtcatct	#163	AB087227				
	blaCMY-1	gaactgacaggcaaacagtgg	cctgccgtataggtggctaa	#40	EF534292				
	blaCMY-2	aagggcattactcggtaggc	cgggataggcgtaactctcc	#23	AF167990				
	blaCTX- M(#1)	aaaaatcactgcgccagttc	cacacttcctaataacagcgtga	#156	EU177100	42.5±2.35			
	blaCTX-M (#2)	tggctcaaaggcaatacga	ttatcacccacagtccacga	#71	EF581888				
	blaIMP	ttaacggttggggtgttgtt	ccgtaaatggagtgtcaatcag	#119	AF290912				

Table 2. Continued.

Class	Gene	Primers		Probe No.	Reference	Alaska	China	Antarctica	Negative control
		F	R						
	blaOXA	tggctggtgggtccttta	gggctaaatggaagcgtttt	#73	EU255288				
	blaSHV	gaacagctggagcgaaagat	gaccggcgagtagtccac	#18	AM176552				
Chloramphenicol resistance	*cat1(#1)*	agttttatccggcctttattca	ggaattccggatgagcatt	#137	V00622	34.5±1.04	35.2±2.03	35.1±1.83	34.5±1.40
	cat1(#2)	ggaattccggatgagcatt	agttttatccggcctttattca	#137	BX664015	35.9±1.76	35.4±1.48	35.1±1.29	35.2±2.03
	cat2(#1)	ttattttgccccggttttta	aacaggtaataatacgcggtcac	#18	X53796				
	cat2(#2)	agtgaatttatggcgggcta	tgcgggaacaatgcagta	#121	X53797				
	catA3	gccgctctcagtacaggttc	cgactaataaagcgtgcaaca	#135	AJ271879				
	catA4	tcaagtgcatcatgccgtat	catcgcagtattgctgtatttca	#135	M11587				
	catA6	agtgaacgatgaacttggttactg	cgtctttgtttctttatttagaatcgt	#58	K00544				
	catA9	tggacttcatttactgggtttaact	tgatgtacctgtaaagatagcggtaa	#18	V01277				
	catA10	aactggagggaagtattcacaagta	taggcatgatgcattttgga	#96	AY238971				
	catA12	tttagaacggcaattaatcagga	atagctgggtatcatctcgtcat	#48	X74948				
	catA14	gtgaggaacagggactgacc	gcttttgcactatgctgttagg	#126	S48276				
	catA16	cgacggttatcatataagcagattt	ccatgtctcataatttgacgctaa	#98	M55620				
	catB1	ccacagtttcgaggattgc	cagaagcttccgatcaccag	#62	M58472				
	catB2	gcgtttgcaaaatcagtcg	aaccgatccacacatcacttc	#151	AJ879564				
	catB3B4	agaagatcaaagcggcaatg	acttgtgcaggccaacaata	#99	gi3393047				
	catB5B8	caaatatcagagtaggccggtatag	tcaaatgagtgcccgtgata	#106	AY123251				
	catB6	tcggcaatgatgtctggata	tattaccgcgccatctcc	#148	AJ223604				
	catB7	ctatttcgagagccccttca	gtatgttcgggttgctgacc	#108	AF036933				
	catB9	tgtggaaatagcgatgttacttg	actctctttcaaccacgattctg	#31	AF462019				
	cml(E-5)	aaccttcgcgagcttcac	gcgacagagatccacaggtc	#162	X59968				
	cmlA	tggggagtgtgatacccaag	aggcatcccattcccatt	#117	M22614				
	cmlB	cagctttgcaatggtcacaa	tgtacaaccagaagttaaggtgct	#38	AF034958				
	cmlV	gcctccaccgtcttcctc	ggaggaaggtcagcacgat	#81	U09991				
	cmrA	atctacgtcctcgggcttg	gagcccggacaacatgaa	#37	AF015087		43.8±2.65		
	cmx	accataccgactgcgaatg	cgaccgaacttgacgtctc	#40	AF024666				
	floR	ccggtatgggcaccttct	cttggcctatgagcacacg	#143	AF118107				
Macrolide resistance	*ereA*	tggagaacgaccagaacactt	gaatctcaaactcattgaagtcca	#90	EF207719				
	ereB	accaatgggccaaagactta	ggctgcagtatgcccactat	#9	AB207867				
	ermA(#1)	gcaacgagctttgggtttac	ggcttaggatgaaaatatagtggtg	#22	BX571856				
	ermA(#2)	gtcgactggtgcctgacc	tagtcacccgtccgcttg	#3	M11276				
	ermC	aaacgctcattggcattatttt	tgaaaatattctcttggaaccatactt	#29	EF526066				
	ermM	aacgctcattggcattactttt	gatgaaaatattctcttggaaccatac	#29	U82607				
	ermML	gcgctccagttgttgaaga	tccgctgagagatctttgg	#153	AF462611				

Table 2. Continued.

Class	Gene	Primers		Probe No.	Reference	Alaska	China	Antarctica	Negative control
		F	R						
	ermTR	cccaacgagctttaggtttg	gaaaatatgctcgtggcacttt	#31	AF002716				
	mefA/E	aggatctgcgatggtcttgt	cccaaaatcgcatagggtaa	#135	AY422727				
	mefE (S. pyogenes)	tcagtctctagaaagtgtcataggtgt	cgcttgaaactgtctgcaat	#22	NC_008022				
	mph	ttcgtggtgaacgacaagc	atctggccacgacgaatc	#87	U34344				
	msrA/B	tgcaaatggtgtaggtaagacaa	ttaggagaacaatcaattccctct	#119	EF092840	41.8±4.84			
Tetracycline resistance	*tet(A)*	cgctttgggtcattttcg	gaaatgccgatcgtggtc	#118	Y19119				
	tet(C)	gtgctcaacggcctcaac	tctcccttatgcgactcctg	#26	EU751612	35.7±0.35	35.3±2.16	37.0±0.41	35.3±6.65
	tet(D)	tgtatccggtcctgatcctg	cagagataatgccctgcaatg	#70	AB089602	43.6±4.69			
	tet(E)	ggcgtaatggtcggtgttt	tccttgaaacagagcgtgtg	#76	DQ366299	43.1±3.78			
	tet(G)	tgctcatgccccgtttat	atgcaggcaagcaggaac	#70	DQ316142	47.7±0.25			
	tet(K)	tgatttttggtaggttagtacaagga	caaccaccataatcagtgaagg	#84	S67449				
	tet(L)	tagccatgggagaaggagtc	ggaccaatgaatataatgggcta	#31	NC_006976				
	tet(M)	aagtgccgccaaatcctt	ctgcattccacttcccaac	#165	EF605269				
	tet(O)#1	ccagacagcagtgacatctttt	tggcctggcgtatctataatg	#88	EF092837				
	tet(O)#2	actgttgccgcagagaaaat	ttactgcaatcgttgtttgga	#55	AY660532				
	tet(Q)	gggctgaaccttgtttgatt	ggacggaggatttgagagc	#156	U10437				
	tet(S)#1	tggcagatgtataccgttcatt	ccatcttttgcagagattagca	#58	DQ377341				
	tet(S)#2	ccatcttttgcagagattagca	tggcagatgtataccgttcatt	#58	EF092839				
	tet(W)	acgctgtcagggatggtatc	ttacgttccagccgaacaa	#19	EU434755				
	tet(X)	agggcagggagtaaatagtgg	ccatcggctagattatcagaca	#138	DQ910317				
Glycopeptide resistance	*vanA(#1)*	aaatcccagcgcatgttc	cagcgacttaagccacttcc	#136	AB186052				
	vanA(#2)	ttataaccgttcccgcagac	ttttgccgtttcctgtatcc	#82	X56895				
	vanB	aacggtgtatggaagctatgc	agtatggcggggagactgt	#135	AY655711				
Positive control	*16S(241–435)*	ccctagctggtctgagagga	cgtaggagtctggaccgtgt	#5		22.6±0.24	25.9±0.29	28.5±0.13	45.3±13.42

Values are cycle numbers for crossing point of amplification curve as determined by the second derivative maximum method. Mean and standard deviation are shown when signals were detected at least twice for triplicate analyses. Selection of primer sites and suitable probe site were done at Universal Probe Library Assay Design Center of Roche Applied Science. Light Cycler® 480 Taqman Probe Master® was used to amplify the antibiotic genes. Details, see text.

of the presently detected genes may help the source-tracking survey on the antibiotic resistance gene in the glacial environment.

In considering the nature and origin of antibiotic resistant genes, many of them are naturally harbored by antibiotic-producing organisms; it is not appropriate to regard that the detection of antibiotic resistant genes in the natural environment is totally attributable to medical and veterinary practices and the use of agrochemicals in crop production. The detection of genes such as *aph(6)* or *ampC* in this experiment may be the case, because *aph(6)* was found in a streptomycin producer (Distler et al., 1987) and chromosomal *ampC* was detected in many gram-negative bacteria (Normark et al., 1986). However, in this context, the predominant detection of *Actinobacteria* and the absence of *aph(6)* in Rink Crags ice cap seem to be contradictory. The predominant detection of proteobacteria and the absence of *ampC* in the same sample is also contradictory. This is a typical constraint for molecular-basis environmental study. Physiological determination on the isolates is of importance to resolve this type of contradiction.

In conclusion, since the number of detected antibiotic resistance genes was larger for Gulkana Glacier samples (8 genes) compared to Ürümqi Glacier (3 genes) and Rink Crags ice cap (0 gene), Gulkana Glacier in Alaska may be the glacier most affected by human industrial activities. Virtually no antibiotic resistant genes were detected from the Antarctic surface ice sample indicating that the "contamination" was still small compared to those in the glacier ice located in the northern hemisphere and not so separated from human industrial activities.

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