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Psychrotrophic Lipase Producers from Arctic Soil and Sediment Samples

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Abstract

Culturable microorganisms were successfully isolated from soil and sediment samples collected in 2011 on the northern coast of Hornsund, West Spitsbergen. A total of 63 single colony isolates from three sampling sites obtained were subjected to temperature dependence study to assess whether they are obligate psychrophilic or psychrotrophic strains. From initial temperature screening, only 53 psychrotrophic isolates were selected that are capable of growing between 4–28°C. The rest that were capable of tolerating higher temperatures up to 37°C were not included in this study. These isolates were chosen for lipase enzyme screening confirmation with the standard plate assay of olive oil and fluorescent dye Rhodamine B. Six lipase positive isolates were also subjected for subsequent lipase enzyme plate screening on tributyrin, triolein, olive oil and palm oil agar. Lipase production by these six isolates was further assayed by using colorimetric method with palm oil and olive oil as the substrate. These isolates with promising lipase activity ranging from 20 U/ml up to 160 U/ml on palm oil and olive oil substrate were successfully identified. Molecular identification by using 16S rRNA revealed that five out of six isolates were Gram-negative *Proteobacteria* and the other one was a Gram-positive *Actinobacteria.*

Key words: Arctic soil, lipase, psychrotroph

Introduction

Polar ecosystems are extensively prospected for obligate psychrophilic and psychrotrophic microbes in order to discover the unique adaptabilities of their enzymes (de Pascale *et al.*, 2012). It is evident that these ongoing research have yield fruitful discoveries of varieties of microbial communities capable of producing cold-active enzymes. In the Arctic region, several bioprospecting studies had led to the findings of a diverse range of bacteria capable of producing cold-active enzymes. A study of bacterial diversity from sediment samples along a transect from the snout of the Arctic Midtre Lovenbreen glacier up to the melt water streams uncovered 32 groups of bacteria, with 14 exhibiting cold-active enzyme activities (Reddy *et al.*, 2009*)*. Sea ice that provides a large pool of organic matters housed several bacterial communities capable of producing cold-active hydrolytic enzymes. Arctic sea ice samples from Canada Basin yield 338 bacterial strains having diverse enzyme producing capabilities, with lipase being the highest (Yu et al. 2009). Sea ice and seawater sampled from Spitzbergen fjords, possessed 116 psychrophilic and psychrotrophic strains; with protease enzyme producers accounted for more than half of the isolated bacteria (Groudieva *et al.*, 2004). A total of 103 bacterial isolates from 47 phylotypes were isolated from eight sediment and one soil samples of two Arctic fjords of Kongsfjorden and Ny-Ålesund, Svalbard with 56% showing either amylase or lipase or both activities (Srinivas *et al.*, 2009). The Finnish Lapland exhibited large annual temperature variations and freeze/thaw events as compared to other Arctic areas. Various ecosystems of Finnish Lapland sampled in Männistö and Häggblom (2006) revealed 331 bacterial strains with protease and lipase producers detected in 59 and 66 isolates.

Extensive research on cold-loving microorganism was carried out because there is a growing prospect of cold-adapted enzymes in the industries. These enzymes were functional at varied and demanding

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industrial conditions. Cold adapted enzymes are in favor because of their ability to save energy, reduce mesophilic microbial contamination and the ease of mild heat-inactivation to terminate the cold-adapted enzymes catalysis (Groudieva *et al.*, 2004). Lipase catalyze both the hydrolysis and synthesis of ester bonds in long-chain acylglycerol ($C \geq 10$) (Jaeger and Reetz, 1998) resulting in the formation of an alcohol and a carboxylic acid (de Pascale *et al.*, 2008). Most of microbial lipase showed high activity between 30°C and 50°C, while alternatively cold active lipase showed high specific activity at lower temperatures. This caused them to become a preferable choice to suit diverse industrial processes (Mayordomo *et al.*, 2000). Due to their interesting property of high activity at low temperatures, production of frail compounds such as synthesis of chiral intermediates would make cold-active lipase as their prime choice. Structural modification in cold-active lipase by an increased flexibility of the polypeptide chain due to higher proportion of amino acids (de Pascale *et al.*, 2012) ease substrate accommodation at low temperatures (Joseph *et al.*, 2008). Cold-active lipase is also reported to work under low water condition due to its high flexibility. In the industry, reverse hydrolysis of substrates in low water conditions are preferable because they are able to lower down water activity besides having improved yields (Tutino *et al.*, 2009).

Besides the need of finding novel cold-active lipase that possess industrial applications, based on protein database from (http://www.ncbi.nlm.nih.gov/protein), least numbers of cold-active lipase structures have been solved. Structure-function and catalytic activity studies of cold-active lipase will be able to help the scientific community in understanding cold adaptation. This will also lead to steps in designing and engineering lipase for specific applications in the future. Therefore, in this study, we attempt to search and identify the lipase producing ability of bacterial isolates from Arctic that are capable of producing cold-active lipase enzyme.

Experimental

Materials and Methods

Study area and sample collection. The field survey and sampling for this study took place during the 2011 boreal summer (August 2011) on the northern coast of Hornsund, Wedel Jarlsberg Land, West Spitsbergen in the vicinity of the Polish Polar Station "Hornsund" (77°00'04"N, 15°33'37"E). Climate of this area is strongly affected by oceanic influence. However, climatic conditions are very variable and characterized by low temperature, low precipitation and strong foehn winds. The average annual air temperature is – 4.4°C, with average monthly temperatures ranging between – 11.3°C in January and + 4.4°C in July, and average annual precipitation reaches only *ca.* 300–400 mm (Marsz and Styszyńska 2007).

A total of twelve sampling locations representing a range of habitats with a diversity of soil physical and chemical characteristics were sampled (data not shown). In this preliminary study, only three sample sites described in Table I were analyzed for this work. Isolates were named according to sites where they were sampled; isolate from soil sample collected at the site A were named ARA(I), isolates from sediment sample collected at the site A were named as ARA(II), and isolates from soil samples collected at the site B and C were named ARB and ARC respectively.

At each sampling location, 10 g of soil or sediment was obtained covering the surface to 10 cm depth using a sterile spatula. As for site C, soil from the bottom of the pond was sampled. The samples were collected with using a sterile spatula and placed into sealed sterile Falcon tubes. Then the samples were refrigerated at 4°C, and subsequently transported at this temperature, taking 6 days in transit to the National Antarctic Research Center, Kuala Lumpur, Malaysia, where they were stored at –20°C until further analysis (Ali *et al.*, 2013).

Table I
Details of sampling locations in the Hornsund area where soil and sediment samples were collected (after Ali *et al.*, 2013).

Sites	Designation	Location	Habitat	Air temperature (°C)		pH Water	Altitude m asl
				Air	Soil		
Site A (soil)	ARA(I)	Fugleberrgsletta, neighbourhood of the Hornsund Station 77°00'06.00" N 15°31'54.60" E	Vertebrate-influenced pond	4.55	7.00	5.98	11
Site A (sediment)	ARA(II)						
Site B (soil)	ARB	Skjerstranda at the foothill Dried runnel of Trulsenfjellet 77°01'28.50" N 15°12'52.74" E		7.30	9.55	–	23
Site C (soil)	ARC	Ralstranda (southern part), at the foot of Rotjesfjellet, near Revelva river 77°00'24.12" N 15°20'36.54" E	Pond	5.85	8.50	5.24	16

Isolation of culturable bacterial strains. An amount of 1 g of soil and sediment were inoculated into 100 ml of nutrient broth medium and grown at 15°C, 150 rpm for 15 days (Leonov, 2010). After the incubation period, the samples were serially diluted 10^{-6} in sterile cold distilled water and isolated on nutrient agar plates. Single colonies obtained with obviously different macroscopic characteristics were subcultured on fresh nutrient agar plates three times to produce pure colony isolates.

Temperature dependence study. In order to differentiate an obligate psychrophilic and psychrotrophic strain growth, isolates obtained that were in pure colony forms were screened by growing on nutrient agar plates and incubated at three different temperatures which were 4°C (in the refrigerator), 28°C ± 2°C (at room temperature) and 37C (in the incubator). Room temperature fluctuates at ± 2°C during day and night time.

Plate screening for lipase. Confirmation of lipase activity via plate screening was done by using the standard Rhodamine B fluorescent assay. Isolates were grown on Rhodamine B agar (1% v/v olive oil and 10 ml of Rhodamine B stock 0.01% w/v). Lipase production on Rhodamine B medium was monitored by orange fluorescence as seen with UV light at 350 nm. Subsequently, lipase activities on lipase positive isolates were also screened using olive oil (1% v/v) agar, palm oil (0.5% v/v) agar, tributyrin (1% v/v) agar and triolein (1% v/v agar). Halo zones seen were considered as positive for lipase production. Tween 20 (0.1% v/v) was added as inducers to all plates except the ones that used tributyrin as substrate. This is due to the fact that initial plate screening without any inducers' addition failed to detect halo zones in all substrates of triolein, olive oil and palm oil.

Standard curve of free fatty acids. A series of oleic acid mixture with isooctane ranging from 0–1000 µmol was prepared in test tubes. The standard curve of free oleic acids vs. absorbance at 715 nm was determined according to Kwon and Rhee (1986). Standard curve of oleic acid was constructed to determine the lipase activity of each isolate during lipase assay.

Lipase assay. Fatty acids released by six lipase positive isolates as confirmed by plate screening were measured by colorimetric method according to Eltaweel *et al.* (2005) with modification. Cultures that had been grown for 5 days at 15°C with olive oil (1% v/v) or palm oil (0.5% v/v) as the substrate were centrifuged at 4,000 rpm to separate the cells and the extracellular enzymes. An amount of 1 ml of crude extracellular enzymes from the supernatant was mixed with 1.48 ml of 100 mM phosphate buffer pH 7, 0.02 ml of 20 mM $CaCl_2 \cdot 2H_20$ and 2.5 ml of olive oil emulsion in 1% poly(vinyl alcohol)(1:3 v/v). The mixture was incubated for 30 minutes at 15°C with the agitation rate of 180rpm. An amount of 1 ml of 6 N HCl and 5 ml isooctane was added to terminate the reaction. The mixture was hand shaken and allowed to separate for 10 minutes. Finally, 4 ml of the upper isooctane layer which contained liberated fatty acids were transferred to a fresh tube containing 0.2 ml of cupric acetate-pyridine reagent and further mixed by vortexing vigorously until foamed. After half an hour at room temperature, absorbance at 715 nm was determined on the isooctane that contained dissolved free fatty acids. One unit of lipase activity was equivalent to 1 µmole of fatty acid released per ml/min at 15°C.

Morphology and molecular identification. Morphology of the six lipase positive isolates was identified through Gram staining and molecular identification by using 16S rRNA analysis. DNA from each isolate was extracted using modified CTAB method (Sambrook and Russell, 2001). The genomic DNA of each isolate was subjected to amplification of the 16S rRNA gene using 27F and 1492R primer pairs. Sequences obtained were aligned using ClustalW (MEGA 5.10) and subjected to BLAST analysis for species identification.

Results

Isolation of culturable bacteria. A total of 63 single colony isolates were obtained from the three sample sites (Table II), comprising 21 soil isolates from site A (named as ARA(I) isolates), 8 sediment isolates from site A (named as ARA(II) isolates), 27 soil isolates from site B (named as ARB isolates) and finally 7 soil isolates from site C (named as ARC isolates).

Temperature dependence study. All isolates were subjected to temperature dependence study to differentiate whether they are obligate psychrophiles or psychrotrophs. Based on growth temperatures used which was 4°C, 28°C ± 2°C and 37°C, each isolate was grouped to three different groups. Isolates that only grew at 4°C were most probably obligate psychrophiles that cannot tolerate growth temperatures higher than 20°C. Isolates capable of growth at both 4°C and 28°C ± 2°C can be grouped as facultative psychrophiles or psychrotroph that tolerated growing temperatures up to 30°C. However, those that also grew at 37°C tolerated such a huge range of temperatures were not included in this study as the aim was to isolate bacteria that adapt to low temperatures. The isolates designation, growing ability at three different incubation temperatures and colony color were given in Table II.

From Site A, three soil isolates, ARA (I) 6, 15 and 19 were capable of growing at 37°C (in addition to 4°C and 28°C ± 2°C). These isolates were removed from further analyses. This left only 18 soil isolates for further lipase screening process. For some of the isolates, the growth medium turned brown/black during the isolates incubation. However, this characteristic was only recorded

Table II
List of isolates, their temperature dependence and macroscopic characteristics. For details on sampling sites location and characteristics see Table I.

Sampling site and substratum	Isolate	Incubation temperature		
		4°C	28°C ± 2°C	37°C
Site A (soil)	ARA(I) 1	/	/*	–
	ARA(I) 2	/	/*	–
	ARA(I) 3	/	/*	–
	ARA(I) 4	/	/*	–
	ARA(I) 5	/	/	–
	ARA(I) 6	/	/*	/*
	ARA(I) 7	/	/	–
	ARA(I) 8	/	/	–
	ARA(I) 9	/	/*	–
	ARA(I) 10	/	/	–
	ARA(I) 11	/	/	–
	ARA(I) 12	/	/*	–
	ARA(I) 13	/	/	–
	ARA(I) 14	/	/*	–
	ARA(I) 15	/	/*	/*
	ARA(I) 16	/	/	–
	ARA(I) 17	/	/	–
	ARA(I) 18	/	/	–
	ARA(I) 19	/	/*	/*
	ARA(I) 20	/	/	–
	ARA(I) 21	/	/	–
Site A (sediment)	ARA(II) 1	/	/	/
	ARA(II) 2	/	/	–
	ARA(II) 3	/	/	–
	ARA(II) 4	/	/	–
	ARA(II) 5	/	/	/
	ARA(II) 6	/	/	/
	ARA(II) 7	/	/	–
	ARA(II) 8	/	/	–
Site B(soil)	ARB 1	/	/	–
	ARB 2	/	/	–
	ARB 3	/	/	–
	ARB 4	–	–	–
	ARB 5	/	/	–
	ARB 6	/	/	–
	ARB 7	/	/	–
	ARB 8	/	/	–
	ARB 9	/	/	–
	ARB 10	/	/	–
	ARB 11	/	/	–
	ARB 12	/	–	–
	ARB 13	/	/	–
	ARB 14	/	/	–
	ARB 15	/	/	–
	ARB 16	/	/	/

Table II continued

Sampling site and substratum	Isolate	Incubation temperature		
		4°C	28°C ± 2°C	37°C
Site B(soil)	ARB 17	/	/	/
	ARB 18	/	/	–
	ARB 19	/	/	–
	ARB 20	/	/	–
	ARB 21	/	/	–
	ARB 22	/	/	–
	ARB 23	/	/	–
	ARB 24	/	/	–
	ARB 25	/	/	–
	ARB 26	/	/	–
	ARB 27	/	/	/
Site C (soil)	ARC 1	/	/	–
	ARC 2	/	/	–
	ARC 3	/	/	–
	ARC 4	/	/	–
	ARC 5	/	/	–
	ARC 6	/	/	–
	ARC 7	/	/	–

/ Presence of growth – Absence of growth
* Blackening of the agar medium

in soil isolates from ARA (I), but not in the sediment samples of ARA (II). The secretion of the brownish pigment into the liquid and solid medium was analyzed through UV-Vis Spectrophotometer (UV-1800 Shimazdu) within the wavelength range of 300–800 nm with acetone as the blank. The maximum absorbance of light was measured at wavelength 320 nm, which is the wavelength absorbance for UV-B region. This high absorbance in UV-B region as well as the dark color of pigment produced (brown) was similar with the characteristics property of melanin pigment (Sajjan *et al.*, 2010; Dastager *et al.*, 2009). In case of sediment isolates from Site A, three pigmented sediment isolates, namely ARA(II) 1, ARA(II) 5 and ARA(II) 6, were capable of good growth at 37°C (see Table II). They were not included in the lipase screening process as well leaving only five sediment isolates for further analyses.

In site B, three isolates (ARB 16, 17 and 27) were eliminated because of their excellent growth at 37C (see Table II). All three isolates were orange pigmented. Isolate ARB 4 failed to grow after subsequent subculturing. Therefore only 23 isolates were subjected to subsequent screening steps. All isolates from site C were subjected for further lipase screening studies.

Plate screening for lipase. All 53 isolates selected through temperature dependence study underwent plate screening for lipase on olive oil agar plates that used Rhodamine B as the fluorescent dye. Only six iso-

Table III
Lipase plate screening of positive isolates

Isolate	Plate screening			
	Tributyrin agar (1% v/v)	Triolein agar (1% v/v) + Tween 20 (0.1% v/v)	Olive oil agar (1% v/v) + Tween 20 (0.1% v/v)	Olive oil agar (1% v/v) + Tween 20 (0.1% v/v)
ARA(I) 5	+	+	+	+
ARA(II)8	+	+	+	+
ARB1	+	+	+	–
ARB2	+	+	+	–
ARB10	+	+	+	–
ARC1	+	+	+	+

+ Presence of halo zones – Absence of halo zones

lates were tested positive for lipase production which were ARA(I) 5 from Site A, sediment isolate ARA(II)8 from Site A, isolate ARB1, ARB2, ARB10 from Site B, and isolate ARC1 from Site C. Pictures of all the positive plates can be seen in Fig. 1. Their morphology obtained through Gram staining is also given in Fig. 1. Besides, the 16S rRNA blast results were also included.

Based on 16S rRNA analysis, lipase positive isolates were dominated by Gram-negative bacteria and only one Gram-positive (ARB1) was detected to produce lipase. Isolate ARA(I)5, ARB2, ARB10 and ARC1 belong to the Gammaproteobacteria group. Isolate ARA(II)8 which was also a Gram-negative bacterium belongs to the Betaproteobacteria group. Isolate ARB1 was the only Gram-positive isolate belonging to the Actinobacteria phylum, which is a high G+C content bacterium. Low percentage of similarities in some of the isolates suggested that some of the isolates might be new species.

In the plate screening method that was done using tributyrin, triolein, olive oil and palm oil substrates, some of the plates (refer Table III) includes addition of Tween 20 that acted as inducers. Isolate ARB1, ARB2 and ARB10 failed to show the presence of halo zones on palm oil agar plates.

Lipase assay. Lipase production was assayed by using palm oil and olive oil. The results are given in Fig. 2. Fig. 2 shows that four isolates – ARA (I) 5, ARA (II) 8, ARB2 and ARB10 prefer olive oil rather than palm oil as their substrate. ARB1 and ARC1 did not follow this pattern, and these isolates utilize palm oil better than olive oil up to 40% better in ARC1.

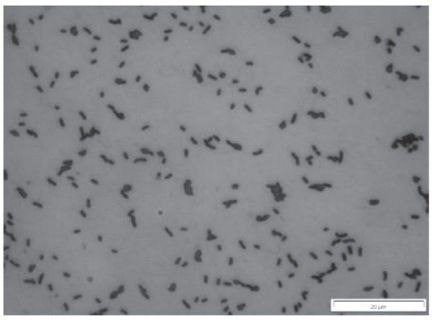
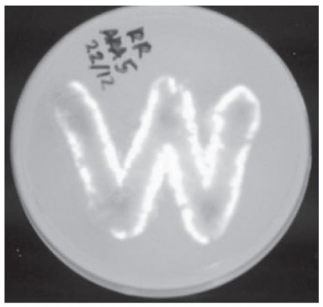
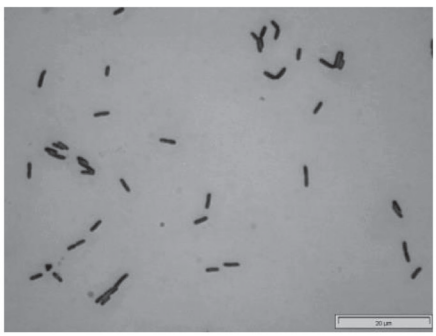
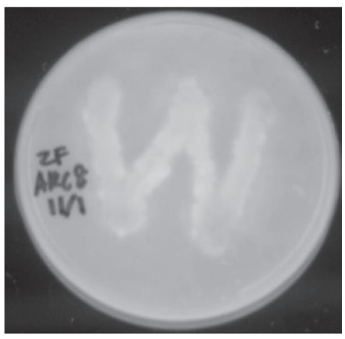
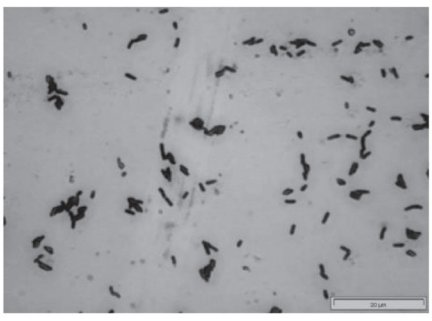
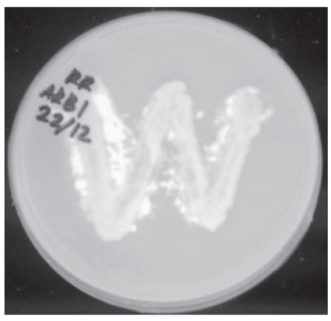
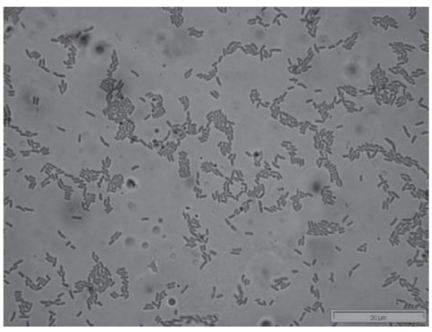
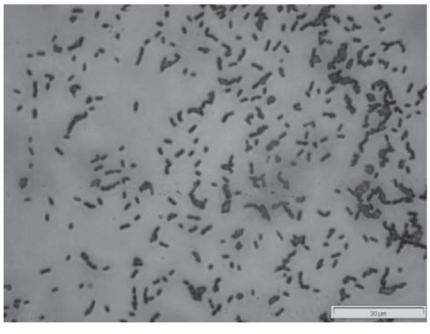
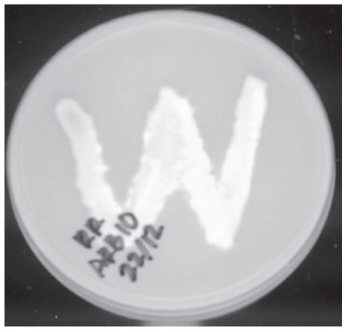
Discussion

Temperature dependence study. In the present study, all of obtained isolates were psychrotrophs. According to Markúsdóttir *et al.* (2013), psychrotrophs can support growth at 0°C, but have an optimal growth between 20°C to 30°C. In the present study, most of the isolates grew faster at incubation temperature 28°C ± 2°C. However, the colonies that grew at 4°C looked healthier. Isolates that are capable of withstanding a wider range of growth temperature are found in environments that experience high seasonal and/or diurnal temperature fluctuations, *e.g.* the sub-Arctic regions (Markúsdóttir *et al.*, 2013). On the contrary, there are also cases of isolates from permanently cold environments that have high proportion of psychrotrophs. For instance, a study on the diversity of culturable bacteria in Arctic sea ice from Spitzbergen fjords revealed that most of the isolates were psychrotrophic rather than obligate psychrophiles (Groudieva *et al.*, 2004). Sampling of the present study was conducted in summer; therefore the thawed layer of soil/sediment would select fast growing organisms capable of exploiting the moderate temperatures and higher nutrient availability (de Pascale *et al.*, 2012).

Plate screening for lipase. Tributyrin was a common substrate to confirm lipase activity in most studies. However, in the present study, other longer chain fatty acids substrates were incorporated for plate screening besides the initial step that used fluorescent dye Rhodamine B to confirm lipase activities. This is due to the fact that shorter chain tributyrin can also be a target of the esterase enzyme. Lipase that possesses a broader substrate range will not only hydrolyze tributyrin, but other longer chain fatty acid substrates such as triolein, palm oil and olive oil. Unlike esterases, lipases are known to attack emulsified form of insoluble long-chain fatty acids substrate (Fojan *et al.*, 2000). This characteristic is a consequence of the 'interfacial activation' phenomenon, where emulsions form caused enhancement of lipase activity (Tutino *et al.*, 2009). Henne *et al.* (2000) also reported that tributyrin positive clones were unable to hydrolyze *p*-nitrophenyl esters during lipase assay. This is a common attribute of the esterase enzyme.

Initially, all isolates screened with olive oil, palm oil agar and triolein agar showed negative results. Therefore, there is a need for inducers presence in the medium. Tweens (polyoxyethylene esters) were found to induce lipase production effectively in *Candida rugosa,* but Tweens themselves do not act as carbon sources (Zhang *et al.*, 2003). Therefore Tween 20 was added as inducers in plate screening with olive oil and palm oil carbon sources in subsequent experiments and this resulted in appearance of halo zones.

Lipase assay. Lipase activity is affected by the carbon length of the fatty acid and the number and position of the double bonds (Long, 2009). Olive oil with the main component of unsaturated oleic acid possess double bonds that can be broken and used easier than palm oil with higher content of saturated palmitic acid that might be harder to break. However, ARB1 and

Isolate	Morphology (Gram staining)	Olive oil agae (with Rhodamine B as the fluorescent dye)
ARA (I) 5 *Pseudomonas brenneri* (99%) (Accession no: gi/219857515/NR_025103.1)	 **Gram-negative rods**	
ARA (II) 8 *Janthinobacterium agaricidamnosum* (94%) (Accession no: gi/219846772/NR_026364.l)	 **Gram-negative rods**	
ARB1 *Arthrobacter sulfureus* (98%) (Accession no: gi/219846645/NR_026237.1)	 **Gram-positive rods**	
ARB2 *Pseudomonas migulae* (98%) (Accession no: gi/219857339/NR_024927.1)	 **Gram-negative rods**	
ARB10 *Pseudomonas lurida* (97%) (Accession no: gi/343201473/NR_042199.1)	 **Gram-negative rods**	

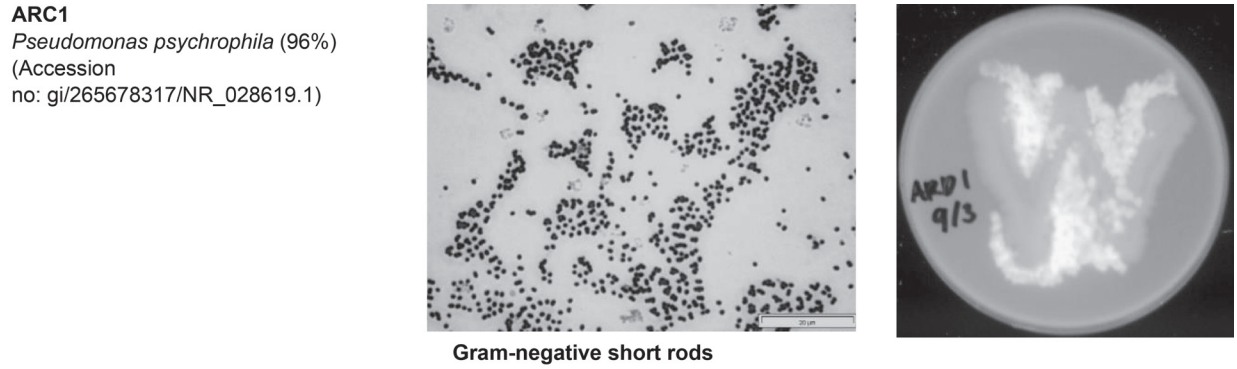


Fig. 1. Lipase positive identified isolates on Olive oil + Rhodamine B agar

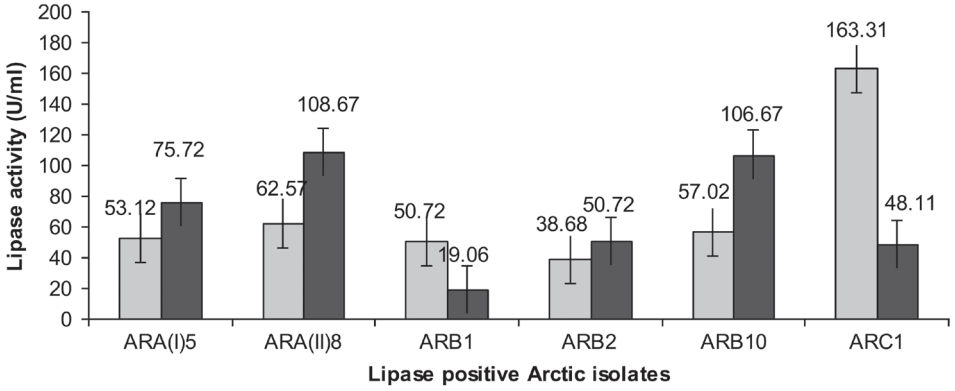


Fig. 2. Lipase activity on palm oil (light shade) and olive oil (dark shade)

ARC1, which grow better on palm oil, probably have some properties that allow easy assimilation of saturated lipids. The nature of that process is unknown and requires further investigations.

Morphology and molecular identification. Most of the work done on bioprospecting at Arctic habitats discovered higher numbers of Gram-negative strains as compared to Gram-positive strains. Srinivas *et al.* (2009) reported the isolation of 38 Gram-negative strains and 9 Gram-positive strains. The low abundance of Gram-positive was due to the fact that low temperature slowed down growth of Gram-positive isolates. The Gram-negative strains in the present study were dominated by pseudomonads. Männistö and Häggblom (2006) also hypothesized that *Pseudomonas* sp. inhibits the growth of certain Gram-negative bacteria.

The resulting isolates group of this present study as determined by 16S rRNA analysis was in agreement with most of bioprospecting work on Arctic environments, where Proteobacteria, CFB (Cytophaga-Flavobacterium-Bacteroidetes) group, low and high G+C Gram-positive genera (Srinivas *et al.*, 2009, Reddy *et al.*, 2009, Groudieva *et al.*, 2004) predominates. In the present study, lipase producing isolates were dominated by Proteobacteria; particularly Gammaproteobacteria where four of the isolates were pseudomonads. Pseudomonads are well known for their metabolic versatility, simple nutrient requirements and genetic adaptability (Markúsdóttir *et al.*, 2013). Most of the pseudomonads from this group bear closest similarities with *Pseudomonas fluorescens* species group, a common isolate found in cold habitats. Meyer *et al.* (2004) reported the isolation of high numbers of *Pseudomonas* sensu stricto genus in the cold alpine Colorado soil, in which *P. fluorescens* group falls into the main cluster.

In the present study, another group of betaproteobacteria, *Janthinobacterium agaricidamnosum* was also found to produce lipase. Tindall *et al.* (2000) isolated two strains of *Janthinobacterium lividum* (clusters 1a and 1b) from a mat in Dry Valleys, Antarctica. *Janthinobacterium agaricidamnosum* isolated from the present study was related to Janthinobacterium *lividum*. These are two well known species in the genus *Janthinobacterium* (Tindall 2004).

Psychrotrophic *Arthrobacter* strains were usually found in the Arctic soils, different Antarctic environments and on various glaciers. They were also abundant in subterranean cave slits (Margesin *et al.*, 2004). Juck *et al.* (2000) reported high G+C Gram-positive cold-adapted microorganisms especially from the genera *Arthrobacter* dominating hydrocarbon contaminated soils; believed to play a role in hydrocarbon degradation.

Strong research interest in cold-active lipase has led to efforts in cloning and expression of respective lipase

genes in the lab currently. Besides that, ecological significance of the lipase enzyme that was present in these six isolates needs further investigation especially on the soil composition of the sampling sites. This is due to the need to discover the source and types of lipids used by these isolates.

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