Bioprospecting around Arctic islands: Marine bacteria as rich source of biocatalysts

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Abstract

We have investigated the biotechnological potential of Arctic marine bacteria for their ability to produce a broad spectrum of cold-active enzymes. Marine bacteria exhibiting these features are of great interest for both fundamental research and industrial applications. Macrobiota water and sediment samples have been collected during 2010 and 2011expeditions around the Lofoten and Svalbard islands. Bacteria were isolated from this material and identified through 16S rRNA gene sequence analysis for the purpose of establishing a culture collection of marine Arctic bacteria.

Herein, we present the functional screening for different extracellular enzymatic activities from 100 diversely chosen microbial isolates incubated at 4 and 20°C. The production of esterase/lipase, DNase and protease activities were revealed in 67%, 53% and 56% of the strains respectively, while 41%, 23%, 9% and 7% of the strains possessed amylase, chitinase, cellulase and xylanase activities, respectively. Our findings show that phylogenetically diverse bacteria, including many new species, could be cultured from the marine arctic environment. The Arctic polar environment is still an untapped reservoir of biodiversity for bioprospecting.

Introduction

High-latitude Arctic oceans and seas have been shown to be important sites for the investigation of marine-derived enzymes. Despite the fact that the Arctic is a region with broad interest as a climate indicator, comparatively little is known about the bacterial diversity [1]. To better understand the microbial communities composition and their sensitivity to environmental change in the Arctic region, recently studies have been conducted on upper sediments and soil [2,3]. In those areas, microorganisms are exposed to several conditions of extreme temperature and high salinity. Microbial adaptations (expressed constantly) such as intracellular processes allow them to thrive or survive in those geochemical polar conditions [4-6]. Enzymes evolve to make this adaptation possible and in a recent study the targeted metagenomics approach has been introduced as promising tool for studying the adaptive evolution of enzymes [7]. The composition of these communities varies by depth, season and location in the ocean. Heterotrophic microbial communities are responsible for a substantial proportion of the main productivity in the ocean due to their role in the marine carbon cycle [8]. In order to avail of this organic matter, members of microbial communities have adapted themselves by producing extracellular enzymes of the correct structural specificity to hydrolyze high molecular weight substrates to small sizes to be transported into

the cell. The ability of microorganisms to produce extracellular enzymes is homogeneously distributed [9-11], but the extent to which enzymatic capabilities change among whole microbial communities in the ocean needs to be largely explored.

The increased interest for cold-active enzymes in academia and research industry is due to their peculiar features such as salt tolerance, high activity at low temperatures in addition to their novel chemical and stereochemical features [12, 13]. The applications of cold-active enzymes are becoming more and more interesting for industry sectors such as consumer products, pharmaceutical, cosmetic, and fine chemicals. Cold-active hydrolytic enzymes can be used in detergents applied for cold washing with a reduction of energy consumption and prevents wear and tear of textile fibers [14]. Other potential applications of cold-active enzymes are evident in processes such as the hydrolysis of lactose in milk by using galactosidase or the taste improvement of refrigerated meat using proteases and betterment of bakery products using glycosidase such as amylases, proteases and xylanases.

Brewing and wine industries use cold-active enzymes as an alternative to warm-active enzymes. The advantage of using cold-active enzymes in food industry is that at low temperatures the risk of contaminations is reduced without destroying the flavor as when high temperatures occur. By this way, it is possible to preserve the nutritional quality of foods. The identification of marine bacteria with the ability to degrade cellulose could lead to improved processes in the quest for cellulosic ethanol [15].

The aim of this work was to investigate the diversity of cultivable Arctic marine bacteria and their extracellular hydrolytic enzymes in the deep-sea sediments and biota samples with the aim to highlight the phylogenetic distribution of the detected activities.

Furthermore, we focused our attention on the extracellular enzymes, produced by the isolated bacteria. It is well established that psychrophilic enzymes, produced by cold-adapted bacteria, display a high catalytic efficiency. This feature is not only important for *in situ* biogeochemical processes, but in particular for their powerful relevance in biotechnological and industrial fields [16,17]. A bioprospecting methodology using functional screening has been performed to explore the extent of microbial enzymatic activity along the coast of the Arctic Svalbard archipelago and Lofoten's islands.

Materials and methods

Two separate scientific expeditions, onboard R/V Helmer Hanssen (University of Tromsø, Norway), were carried out in the Lofoten area (Northern Norway) in April 2010 and on the coastal areas around the Svalbard archipelago in October 2011. Various microbiota (animals and algae), sediment and seawater samples was collected by benthic and pelagic trawls, divers, a van-ween grab and a water sampler. In total, more than 50 stations were sampled between 400 and 70 m water depth with a sea temperature between 1-6°C. Scientist at the University of Tromsø performed the isolation, culturing and preliminary classification of the bacterial isolates. The bacteria are stored in an in-house collection together with all meta-data. The procedures were:

Isolation and culturing of bacteria

The animals and algae were dissected and the tissues and intestinal contents homogenized, diluted and subsequently plated onto marine agar plates. The sediments were dissolved in sterile seawater, centrifuged to remove particles and 100 µl of a dilution series was plated out. A dilution series of 100 µl of the seawater samples was also plated out. All plates were incubated aerobically at 4-12°C. The selective agar plates were: IM5 (humic acid agar, with sea water), humic acid (1 g), K2HPO4 (0.5 g), FeSO4•7H2O (1 mg), agar (20 g), vitamin B solution (1 mL), natural sea water (0.5 L) and distilled water (0.5 L); IM6 glycerol (0.5 g), starch (0.5 g), sodium propionate (0.5 g), KNO3 (0.1 g), asparagine (0.1 g), casein (0.3 g), K2HPO4 (0.5 g), FeSO4•7H2O (1 mg), agar (20 g), vitamin B solution (1 mL), natural sea water (0.5 L) and distilled water (0.5 L); IM7 (chitin agar, with sea water) chitin (Sigma), K2HPO4 (0.5 g), FeSO4•7H2O (1 mg), agar (20 g), vitamin B solution (1 mL), natural sea water (0.7 L) and distilled water (0.3 L); IM8, malt extract (1 g), glycerol (1 g), glucose (1 g), peptone (1 g), yeast extract (1 g), agar (20 g), natural sea water (0.5 L) and distilled water (0.5 L). All isolation media were amended with filtered (0.2-µm pore size) cycloheximide (50 µg/mL), nystatin (75 µg/mL) and nalidixic acid (30 µg/mL) [18]. On land the bacteria was restreaked onto new plates and single colonies were used to inoculate 5 ml cultures. One ml of dense culture was cryo preserved in 20% glycerol, while one ml of culture was harvested by centrifugation using a tabletop centrifuge at 12,000 rpm for 3 min, washed once with 1 ml of distilled water and re-centrifuged

for 3 min. Tubes were afterwards frozen at -20°C for later extraction of genomic DNA.

Genomic DNA extraction and partial sequencing of the 16S rRNA gene

Premixed cold Instagene matrix (BioRad, Hercules, CA), 200 µl, was added to the frozen bacterial pellets and tubes vortexed to dissolve the pellets. Samples were then heated for 30 min at 56°C, vortexed for 10 s and then heated again for 8 min at 100°C with a final vortexing for 10 s. Tubes containing genomic DNA and cell debris were centrifuged for 3 min and approximately 0.5 µl of supernatant was used as template for PCR reactions. To generate a PCR product 0.2 µM of universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT -3′) were combined with 1.5 mM $MgCl_2$, 0.2 mM dNTP mix, 0.5 µl genomic DNA, 1X Taq buffer and 1.25U Taq polymerase in a 0.2 ml PCR tube. Water was added to a final volume of 50 µl. PCR was conducted using an initial denaturation for 3 min at 94°C followed by 30 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 2 min. A final extension step for 7 min at 72°C was performed before cooling to 4°C. Presence of amplified product was checked by running 5 µl on a 1% agarose gel. PCR product was purified using ExoSap-IT (Affymetrix, Santa Clara, CA) or PureLink Pro 96 PCR Purification Kit (Invitrogen-Life Technologies, Carlsbad, CA) following manufacturers protocols. In sequencing PCR about 50-100 ng template was mixed with buffer and 2 µl BigDye v 3.1 together with 1 µl (10 µM) sequencing primer 515F (5′-GTGCCAGCAGCCGCGGTAA-3′). Water was added to a final volume of 20 µl. PCR program was set up according to the BigDye Terminator v 3.1 Cycle

Sequencing Kit protocol (Applied Biosystems, Carlsbad, CA) and further handling was done at the University of Tromsø's DNA sequencing core facility.

Bacterial 16S rRNA gene phylogeny

The ABI2FASTA converter v 1.1.2 (available online) was used to extract FASTA sequence files from ABI output files and low quality ends were trimmed (http://www.dnabaser.com).

The trimmed sequences were then checked for chimeras using DECIPHER's Find Chimeras web tool (http://decipher.cee.wisc.edu/FindChimeras.html). Sequence search against GenBank using BLAST [19] was performed to identify the genus each bacterium belongs to.

The sequences, around 800-900 bp, depending on the isolate, were compared with those data available in the RDPII (Ribosomal Database Project II) to determine the relative phylogenetic positions. Multiple alignments were generated using CLUSTAL W [20]. Alignments were edited using BioEdit Sequence Alignment Editor version 3.0.3 [21] and regions of ambiguous alignment were removed. The sequences were then entered into the MEGA version 5 program [22] to produce a phylogenetic tree. The phylogenetic tree was constructed using the maximum likelihood method [23] with General Time Reversible and complete-deletion model analysis. The resultant tree topologies were evaluated by bootstrap analysis based on 500 replicates. DNA sequences are deposited in GenBank under Accession numbers KF313361-KF313377, KF313380-KF313402, KF313404-KF313463.

Growth Temperature

Bacterial strains were growth in marine 2216 broth (Difco) at two different temperatures according to their classification. Gram-positive were grown at 20°C and Gram-negative were grown at 4°C using a Heidolph tube shaker (Heidolph, Germany) at 600 rpm.

Extracellular enzymatic activities

Protease, esterase/lipase, chitinase, cellulase, gelatinase, amylase, xylanase, DNase were detected on marine agar assay plates. Log-phase bacteria cultures were spotted (10 µl) onto assay plates which were incubated at 4 and 20°C. The appearance of a halo was evaluated after one week.

Screening of protease producing bacteria

Screening of bacteria isolates for protease activity was performed on marine 2216 broth agar supplemented with 1% (w/v) skimmed milk [24]. Skimmed milk was prepared using a 10% (w/v) stock solution of commercially available nonfat milk powder. Marine broth agar was autoclaved at 121°C for 15 min, while 10% (w/v) milk powder solution was autoclaved at 115°C for 10 min.

Exactly 10% (w/v) milk solution was mixed with marine broth agar to a final concentration of 1% (w/v) while still hot. Protease producing bacteria were selected based on the formation of halo zone of clearance around the colony.

Screening of esterase/lipase producing bacteria

Screening of bacteria isolates for esterase/lipase activity was performed on marine 2216 broth agar supplemented with 1% (w/v) tributyrin [25]. Marine broth agar was autoclaved at 121°C for 15 min and 100% (w/v) glyceryl tributyrate solution

(Sigma) was mixed after sonication with marine broth agar to a final concentration of 1% (w/v). Esterase/lipase producing bacteria were selected based on the formation of halo zone of clearance around the colony.

Screening of chitinase producing bacteria

Screening of bacteria isolates for chitinase activity was performed on marine 2216 broth agar supplemented with 0.5% (w/v) colloidal chitin. Colloidal chitin was prepared using commercial chitin (Sigma-Aldrich) from shrimp shells [26]. Marine broth agar was autoclaved at 121°C for 15 min and mixed with 5% (w/v) colloidal chitin solution to a final concentration of 0.5% (w/v). Chitinase producing bacteria were selected based on the formation of halo zone of clearance around the colony. For the visualization of the chitinolytic activity, the agar plates were flooded with 0.5% congo red solution for 30 minutes and destained with 1M NaCl for 20 min [27].

Screening of cellulase producing bacteria

Screening of bacteria isolates for cellulase activity was performed on marine 2216 broth agar supplemented with 1.5% (w/v) carboxymethylcellulose (CMC) low viscosity sodium salt (Sigma). After incubation, the plates were flooded with 0.5% Congo red for 15–20 min followed by destaining with 1 M NaCl for 15–20 min. The extracellular cellulase activity was detected by the presence of clear zone around the growing colony against the dark red background [28].

Screening of gelatinase producing bacteria

Screening of bacteria isolates for gelatinase activity was performed on marine 2216 broth agar supplemented with 0.4% (w/v) gelatin (Sigma) [29]. Marine broth

agar was autoclaved at 121°C for 15 min and sterilized aqueous solution of gelatin (8% w/v) was added to the medium before pouring to obtain 0.4% gelatin concentration in the medium. Gelatinase producing bacteria were selected based on the formation of halo zone of clearance around the colony after staining with Comassie blue (0.25% w/v) in methanol acetic acid-water 5:1:4 (v/v/v) and destained by using methanol and acetic acid. The extracellular gelatinase activity was detected by the presence of clear zone around the growing colony against the dark blue background.

Screening of amylase producing bacteria

Screening of bacteria isolates for amylase activity was performed on marine 2216 broth agar supplemented with 2% (w/v) starch (Sigma). Marine broth agar and starch were autoclaved at 121°C for 15 min. After incubation, the plates were flooded with 0.5% Congo red for 15–20 min followed by destaining procedure with 1 M NaCl for 15–20 min [30]. The extracellular amylase activity was detected by the presence of clear zone around the growing colony against the dark red background.

Screening of xylanase producing bacteria

Screening of bacteria isolates for xylanase activity was performed on marine 2216 broth agar supplemented with 2.5 g/L xylan from beechwood (Sigma) [31]. Marine broth agar and xylan were autoclaved at 121°C for 15 min. After incubation, the plates were flooded with 0.5% Congo red for 15–20 min followed by destaining with 1 M NaCl for 15–20 min. The extracellular xylanase activity

was detected by the presence of yellow zone around the growing colony against the dark red background.

Screening of DNase producing bacteria

The extracellular bacterial DNases were screened using DNase test agar (Merck) and all plates were supplemented with extra 0.3 M NaCl. For the detection of DNase-producer bacterial strains, the plates were flooded with 0.1 M HCl solution. A clear or dim halo around a colony after one week indicated a positive exoenzyme-producing isolate [32].

Results

Bacterial phylogenetic diversity

The phylogenetic diversity of 100 selected arctic bacterial isolates was determined by 16S rRNA gene sequence analysis. Based upon the 16S rRNA gene sequences a phylogenetic tree was constructed to visualize their affiliations (Figure 1). Out of 60 Gram-negative phylotypes, 55 belonged to the phylum *Proteobacteria*, 5 to the phylum *Bacteroidetes.* Out of 40 Gram-positive phylotypes, 31 belonged to the phylum *Actinobacteria* and 9 belonged to the phylum *Firmicutes*. The nearest phylogenetic neighbor, in terms of highest bit score and the sequence identity (%) to known isolates, were also determinate by blast searches using the16S rRNA gene sequences as shown in Tables 1 and 2. Several Gram-positive and Gram-negative isolates can be considered new species as the identity value to known isolates is below 97-98 %. The novel isolates are members of *Filibacter, Leifsonia, Planococcus, Rhodococcus, Streptomyces, Flavobacterium,*

Gelidibacter, Marinobacter, Pseudomonas, Psychromonas, Roseobacter, Roseovarius, Serratia, Shewanella and Thalassospira.

Screening and detection of extracellular enzymatic activities

The bacterial isolates were screened for the production of extracellular enzymes at 4°C and 20°C. We detected extracellular activities of all 100 isolates on marine agar plates. The ability to degrade substrates was tested on tributyrin, skim milk, chitin, carboxymethylcellulose, gelatin, starch, xylan and DNA (Figure 2). 67%, 53% and 56% of the isolates were positive for esterase, DNase and protease activity respectively, while 41%, 23%, 9% and 7% of the isolates showed the ability to produce amylase, chitinase, cellulase and xylanase activity respectively. In tables 3 and 4, the hydrolase enzymatic activities production, in terms of halo size, corresponding to each isolate is shown. This activity overview highlight the multiple enzyme activities mainly localized in the Gram-negative bacterial group. The ability to degrade tributyrin, skim milk and DNA was almost equally distributed among the isolates of *Proteobacteria*, *Bacteroidetes*, *Actinobacteria* and *Firmicutes*. When the same activities were analyzed at 4°C, *Shewanella* SP043 displayed the highest esterase activity while *Pseudoalteromonas*, *Pseudomonas*, *Acinetobacter*, *Roseovarius* and *Psychrobacter* isolates showed a high esterase activity at 20°C. The protease activity was detected at 4 and 20°C. Concerning the activity detected at 4°C, the halo size was bigger than what we observed for the other enzymatic activities. The biggest size of the halo was reached at 20°C when *Shewanella*, *Gelidibacter* and *Pseudoalteromonas* isolates were spotted on the plates. The chitinase activity was detected in members of

Proteobacteria such as *Pseudomonas, Photobacterium* and *Serratia*, which indicate that these bacteria may be important degraders of chitin in the marine sedimentecosystem and contribute to the recycling of vital carbon and nitrogen resources. At 20°C, the best chitinase activity was detected in *Brevundimonas* sp. while a lower activity was observed for *Pseudomonas, Roseobacter*, *Shewanella, Photobacterium and Serratia.* At 4°C, the best chitinase activity was observed in *Serratia* sp. The functional screening for chitinases by Gram-positive bacteria gave only negative results, in terms of halos. The xylanase activity was exclusively detected by the members of *Promiconospora*, *Serratia*, *Pseudoalteromonas* and *Clavibacter*. In our experiments large halos was observed at 20°C for the *Serratia* and *Promiconospora* isolates. A very low activity, sometimes faint, was displayed at 4°C for the Gram-negative bacteria. *Clavibacter* was the only Gram-positive bacteria that showed xylanase activity.

The gelatin and starch were more hydrolyzed by Gram-negative bacteria. The best gelatinase activity was detected for the *Brevundimonas* strain at 20°C. *Pseudoalteromans* showed the best amylase activity at 20°C compared to *Serratia, Flavobacterium, Photobacterium* and *Psychrobacter*.

A few Gram-negative isolates were able to produce extracellular cellulases. The cellulase activity was detected among the isolates of *Proteobacteria*. Cellulase-positive isolates were members of *Serratia*, *Photobacterium*, *Moritella* and *Pseudoalteromonas*. No cellulase activity was identified from the functional screening performed for the Gram-positive bacteria at 20°C.

Discussion

A collection of 354 bacteria had been obtained during two separate research cruises to the Lofoten area (Northern Norway) and around the Svalbard archipelago.100 bacterial isolates were chosen based on the differences at the 16S rRNA gene nucleotidic sequence level compared to other isolates belonging to the same genus. The growth temperature range for 100 selected isolates was tested on marine 2216 broth and all the isolates were able to grow in this medium without any additional requirement of sodium chloride or seawater. The result pointed out the ability of these isolates to survive and proliferate in the low temperature and constant salinity. We noticed however that the Gram-positives, in general, needed longer time at low temperatures to reach the same colony size than the Gram-negative bacteria. A functional-based screening method was performed to detect the extracellular enzyme activities. A suitable concentration of substrate was chosen to decrease possible toxic effects for bacteria growth. Hydrolytic activities were easily detected on tributyrin and skim milk plates. All other activities that required a staining/post-staining experimental step were detected after an optimization of the already known protocols according to the visible halos on the marine broth agar plates. The size of halos was used to compare enzymatic activities.

It is worth noting that in the functional screenings carried out, sometimes bacteria that had highly similar 16s rRNA gene sequences, isolated from different cold environments, displayed different behavior referring to the same enzymatic activity. A comparison of those arctic bacterial isolates that might be novel species with previously published species, in terms of detected enzymatic

activities, was performed. To our knowledge, for members of the genera *Planococcus* and *Rhodococcus* there have not been reported any amylase or alginate lyase activities respectively so far.

Environmental temperature not only has effects on microbial activity, but can also affect activity indirectly by changing the temperature dependency of the whole community [23]. This is additional evidence of the prodigious and extraordinary ability of the bacteria to adapt themselves and to develop new strategies to survive in such extreme and changeable microenvironment and community composition.

The experimental approach could be considered propaedeutic in order to introduce in the industrial market new psychrophilic and psychrotolerant enzymes produced by a wide range of isolated Arctic bacteria. Sometimes there is a difficult in growing bacterial isolates in the laboratory and an essential prerequisite for biodiscovery fails [34,35]. The high number of bacterial isolates allowed us the opportunity to explore the connection between extracellular activity and biodiversity in the Arctic regions. The cold-active enzymes activities detected in this study indicated that many Arctic bacteria are able to hydrolyze the major constituents of the organic matter such as esters, proteins, α-and β-linked polysaccharides. These features make these hydrolytic enzymes potential biocatalysts for use in several industrial fields [36,37]. Studies have revealed the strong potency of cold-active lipases for the organic synthesis of valuable short-chain esters such as flavors used in food and pharmaceuticals [38]. Amylases are essential in the conversion of starches into oligosaccharides and they are used in the production of maltodextrin, glucose or fructose syrups and in different sectors

like textile, paper and detergent industries [39]. Xylanases and cellulases are described as potential biocatalysts for bioethanol production but also useful in paper and pulp industry, agriculture and food/feed industry [40,41]. Chitinases are required in industry to solve problems like waste decomposition or biocontrol agent for insects in agriculture [42]. Proteases are a large group of enzymes present in bioindustry in detergents, food, metal recovery and waste treatment sectors [43].

Nowadays, it is also becoming promising to engineer the microbes and whole bacterial communities for direct conversion of biomass or substrates degradation avoiding laborious production and purification steps [44-46]. In this respect, the arctic marine bacteria collection presented in this work may play and important role and open new perspective in the field of microbial biodegradation engineering.

Concluding remarks

This work provides insight into the microbial diversity that populates the Arctic region and further shows the vast genetic potential of these “cold-loving” microorganisms to produce hydrolytic enzymes that can be fed into the bio-based economy.

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Conflict of Interest

The authors declare no conflict of interest.

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Figure 1. Phylogenetic tree showing the relationships among the arctic marine bacterial isolates based on 16S rRNA gene homology. Reference sequences of type strains (●). GeneBank accession numbers of 16S rRNA gene sequences are shown in parentheses. Gram-positive and Gram-negative bacteria are shown with blue and black branches. The taxonomic classifications are shown with different colored squares: magenta (Actinobacteria), green (Firmicutes), brown (Flavobacteria) and red (Proteobacteria).

Figure 2. Functional screening of marine bacteria on marine agar plates for: (**A**) protease activity; (**B**) esterase/lipase activity (**C**) chitinase activity; (**D**) amylase activity; (**E**) xylanase activity; (**F**) gelatinase activity; (**G**) DNase activity; (**H**) cellulase activity.

Table 1. Nearest phylogenetic neighbor (highest bit score) and identity (%) by 16S rRNA gene analysis of 40 Gram-positive marine bacteria isolates. The length of 16S rRNA gene sequences associated with its best hits is roughly 800-900 bp. The identity (%) values of those isolates that might be considered new species are shown in bold.

GRAM +		Phylogenetic neighbor	Bit-Score	Ident. (%)	Accession number
Arthrobacter AW25M11		*Arthrobacter antarcticus SPC26*	1585	99	NR 115079.1
Arthrobacter AW19M24		*Arthrobacter bergerei Ca106*	1530	99	NR 025612.1
Arthrobacter	GB04J08	*Arthrobacter cryoconiti Cr6-08*	1507	99	NR 108846.1
Arthrobacter	SP003	*Arthrobacter siccitolerans 4J27*	1345	99	NR 108849.1
Bacillus AW25M04		*Psychrobacillus psychrodurans 68E3*	1576	99	NR 025409.1
Bacillus	GB04J31	*Sporosarcina psychrophila (Bacillus psychrophilus) NBRC 15381*	1604	99	NR 113752.1
Clavibacter	SP011	*Clavibacter michiganensis LMG7333*	1452	99	NR 118300.1
Clavibacter	SP033	*Clavibacter michiganensis ATCC 33566*	1264	99	NR 118871.1
Filibacter	KH04J17	*Filibacter limicola*	1563	**98**	NR 042024.1
Filibacter AW28M30		*Filibacter limicola DSM 13886*	1589	**98**	NR 042024.1
Knoellia	SP073	*Knoellia subterranean HKI 0120*	1430	99	NR 028939.1
Leifsonia	SP050	*Rhodoglobus aureus (Leifsonia aurea) CMS 81*	438	99	NR 028013.1
Leifsonia	AW28M06	*Salinibacterium amurskyense KMM 3673*	1365	**98**	NR 041932.1
Leifsonia	AW02J23	*Leifsonia rubra CMS 762*	1463	**98**	NR 028012.1
Microbacterium AW28M07		*Microbacterium lacus A5E-52*	1535	99	NR 041563.1
Microbacterium AW28M15		*Microbacterium hydrocarbonoxydans BNA48*	1561	99	NR 042263.1
Microbacterium	SP006	*Microbacterium maritypicum DSM 12512*	1408	100	NR 114986.1
Micrococcus	AW19M49	*Micrococcus yunnanensis YIM 65004*	1439	99	NR 116578.1
Micrococcus	SP063	*Micrococcus yunnanensis YIM 65004*	1096	100	NR 116578.1
Nesterenkonia	AW19M55	*Nesterenkonia lutea YIM 70081*	1406	99	NR 029120.1
Nocardiopsis	BA19M08	*Nocardiopsis prasina DSM 43845*	1435	99	NR 044906.1
Planococcus	AW02J18	*Planococcus citreus NBRC 15849*	1428	99	NR 113814.1
Planococcus	GB02J13	*Planococcus halocryophilus Or1*	1498	**98**	NR 118149.1
Plantibacter	AW25M38	*Plantibacter auratus IAM 14817*	1533	99	NR 041045.1
Pseudomonas	AW19M56	*Pseudomonas brenneri CFML 97-391*	1574	99	NR 025103.1
Psycrobacillus	AW28M34	*Psychrobacillus psychrodurans 68E3*	1543	99	NR 025409.1
Rhodococcus	AW19M46	*Rhodococcus erythropolis PR4*	1581	99	NR 074622.1
Rhodococcus	AW25M09	*Rhodococcus fascians CF17*	1397	**98**	NR 037021.1
Rhodococcus	GB23J02	*Rhodococcus yunnanensis YIM 70056*	1585	99	NR 043009.1
Rhodococcus	MP02J07	*Rhodococcus yunnanensis YIM 70056*	1592	**98**	NR 043009.1
Rhodococcus	SP061	*Rhodococcus cerastii C5*	455	100	NR 117103.1
Rhodococcus	SP062	*Rhodococcus trifolii T8*	1387	**98**	NR 108505.1
Salinibacterium	SP028	*Salinibacterium amurskyense KMM 3673*	1450	99	NR 041932.1
Sanguibacter	SP022	*Sanguibacter antarcticus KOPRI 21702*	1387	99	NR 044173.1
Staphylococcus	AW02J12	*Staphylococcus saprophyticus ATCC 15305*	1432	99	NR 074999.1
Staphylococcus	SP052	*Staphylococcus equorum PA231*	1452	100	NR 027520.1
Streptomyces AW19M35		*Streptomyces fulvissimus DSM 40593*	1592	99	NR 103947.1
Streptomyces	BA19M03	*Streptomyces clavifer NRRL B-2557*	1646	99	NR 043507.1
Streptomyces	AW19M42	*Streptomyces laculatispora BK166*	1548	**98**	NR 117082.1
Tomitella	SP012	*Tomitella biformata AHU1821*	1419	99	NR 112905.1

Table 2. Nearest phylogenetic neighbor (highest bit score) and identity (%) by 16S rRNA gene analysis of 60 Gram-negative marine bacteria isolates. The length of 16SrRNA sequences associated with its best hits is roughly 800-900 bp. The identity (%) values of those isolates that might be considered new species are shown in bold.

GRAM -		**Phylogenetic neighbor**	**Bit-Score**	**Ident. (%)**	**Accession number**
Achromobacter	AW28M02	*Achromobacter piechaudi NBRC 102461*	1572	99	NR 114102.1
Achromobacter	GB02J42	*Achromobacter spanius CCUG 47062*	1467	99	NR 118402.1
Acinetobacter	GB02J46	*Acinetobacter johnsonii ATCC17909*	1500	99	NR 117624.1
Brevundimonas	KH11J01	*Brevundimonas vescicularis NBRC 12165*	1471	99	NR 113586.1
Flavobacterium	SP045	*Flavobacterium frigoris NBRC 102678*	1439	**98**	NR 112816.1
Flavobacterium	SP046	*Flavobacterium degerlachei NBRC 102677*	1245	99	NR 112815.1
Gelidibacter	GB04J26	*Bizionia paragorgiae KMM 6029*	1471	**98**	NR 025827.1
Halomonas	R5-57	*Halomonas glaciei*	1676	99	NR 114866.1
Halomonas	SP051	*Halomonas neptunia Eplume 1*	1459	100	NR 027218.1
Marinobacter	CK-1	*Marinobacter antarcticus*	2494	**97**	NR 108299.1
Marinomonas	SP036	*Marinomonas primoryensis NBRC 103029*	1489	100	NR 114182.1
Mesorhizobium	SP068	*Hoeflea alexandri AW1V30*	1290	99	NR 042321.1
Moritella	SP016	*Moritella marina ATCC 15381*	1426	99	NR 040842.1
Oceanisphaera	SP013	*Oceanisphaera ostreae T-W6*	1448	99	NR 109099.1
Photobacterium	GB02J53	*Photobacterium phosphoreum NBRC 103031*	1568	99	NR 114184.1
Photobacterium	SP001	*Photobacterium phosphoreum NBRC 103031*	1502	100	NR 114184.1
Photobacterium	SP044	*Photobacterium indicum NBRC 14233*	1339	99	NR 112225.1
Photobacterium	SP005	*Photobacterium phosphoreum NBRC 103031*	1448	100	NR 114184.1
Polaribacter	KH04J14	*Polaribacter butkevichii KMM 3938*	1502	99	NR 042779.1
Polaribacter	SP072	*Polaribacter sejongensis KOPRI 21160*	1406	99	NR 109324.1
Promiconospora	AW19M33	*Promiconospora vindobonensis V-45*	1513	99	NR 042146.1
Pseudoalteromonas	AW25M26	*Pseudoalteromonas Antarctica NF3*	1615	99	NR 029317.1
Pseudoalteromonas	AW28M34b	*Pseudoalteromonas haloplanktis TAC 125*	2737	99	NR 102834.1
Pseudoalteromonas	GB02J33	*Pseudoalteromonas larrageenovora NBRC 12985*	1576	99	NR 113605.1
Pseudoalteromonas	SP007	*Pseudoalteromonas translucida KMM 520*	1478	99	NR 025655.1
Pseudoalteromonas	SP077	*Pseudoalteromonas haloplanktis TAC 125*	1408	99	NR 102834.1
Pseudomonas	AW28M04	*Pseudomonas anguilliseptica S1*	1506	99	NR 029319.1
Pseudomonas	GB04J27	*Pseudomonas ludensis ATCC 49968*	1465	99	NR 024704.1
Pseudomonas	AW25M15	*Pseudomonas brenneri CFML 97-391*	1522	99	NR 025103.1
Pseudomonas	KH04J19	*Pseudomonas brenneri CFML 97-391*	1531	**98**	NR 025103.1

Table 2. Cont.

GRAM -		Phylogenetic neighbor	Bit-Score	Ident. (%)	Accession number
Psychrobacter	AW25M27	*Psychrobacter cryohalolentis K5*	1622	99	NR 075055.1
Psychrobacter	GB04J30	*Psychrobacter piscatorii T-3-2*	1531	99	NR 112807.1
Psychrobacter	SP009	*Psychrobacter celer SW-238*	1341	99	NR 043225.1
Psychrobacter	SP042	*Psychrobacter namhaensis SW-242*	1677	99	NR 04 3141.1
Psychromonas	SP017	*Psychromonas arctica Pull 5.3*	1362	99	NR 028821.1
Psychromonas	SP041	*Psychromonas arctica Pull 5.3*	1236	**98**	NR 028821.1
Rhodobacter	AW25M51	*Gemmobacter changlensis JA 139*	1561	99	NR 042564.1
Roseobacter	GB02J23	*Celeribacter baekdonensis L-6*	1585	100	NR 117908.1
Roseobacter	GB02J24	*Tropicibacter mediterraneus M17*	1487	**98**	NR 125557.1
Roseobacter	AW25M03	*Celeribacter baekdonensis L-6*	1459	99	NR 117908.1
Roseobacter	AW19M09	*Celeribacter baekdonensis L-6*	1493	99	NR 117908.1
Roseovarius	GB02J02	*Pseudoruegeria lutimaris HD-43*	1212	**96**	NR 116620.1
Serratia	GB02J45	*Serratia plymuthica A59*	1511	**98**	NR 102827.1
Serratia	SP010	*Seratia proteamaculans DSM 4543*	1295	99	NR 025341.1
Shewanella	KH04J08	*Shewanella surugensis C959*	1273	99	NR 040950.1
Shewanella	AW25M33	*Shewanella frigidimarina NCIMB 400*	1535	99	NR 074814.1
Shewanella	MP02J10	*Shewanella halifaxensis HAW-EB4*	1459	99	NR 074822.1
Shewanella	SP023	*Shewanella hanedai NBRC 102223*	1389	**98**	NR 114050.1
Shewanella	SP035	*Shewanella gelidimarina ACAM 456*	1343	99	NR 026058.1
Shewanella	SP043	*Shewanella piezotolerans WP3*	1243	99	NR 074738.1
Sphingopyxis	GB02J19	*Sphingopyxis flavimaris SW-151*	1522	99	NR 025814.1
Stenotrophomonas	AW25M54	*Stenotrophomonas rizophila e-p10*	1583	99	NR 121739.1
Stenotrophomonas	AW25M14	*Stenotrophomonas chalatiphaga LPM-5*	1517	99	NR 116366.1
Sulfitobacter	SP069	*Sulfitobacter litoralis Iso3*	1393	100	NR 043547.1
Sulfitobacter	AW25M05	*Sulfitobacter marinus SW-265*	1552	99	NR 043936.1
Thalassospira	AW2545	*Thalassospira lucentensis QMT2*	1531	99	NR 115011.1
Thalassospira	AW19M11	*Thalassospira lucentensis QMT2*	1458	**97**	NR 115011.1
Thalassospira	KH04J01	*Thalassospira lucentensis QMT2*	1493	**98**	NR 115011.1
Thalassospira	BA19M05	*Thalassospira lucentensis QMT2*	1544	99	NR 115011.1
Vibrio	SP025	*Vibrio toranzoniae Vb 10.8*	1352	99	NR 117680.1

Table 3. Hydrolase activities of 40 Gram-positive marine bacterial isolates from Lofoten and Svalbard. Halo size is indicated by: (+) small halo (3 mm), (++) medium halo (5 mm), (+++) large halo (10 mm) and (-) no indication of halo.

		Alginate lyase	Amylase	Cellulase	Chitinase	DNase	Gelatinase	Lipase	Protease	Xylanase
GRAM +		20°C	20°C	20°C	20°C	20°C	20°C	20°C	20°C	20°C
Arthrobacter	AW25M11	-	-	-	-	-	-	+	-	-
Arthrobacter	AW19M24	-	++	-	-	-	-	-	+	-
Arthrobacter	GB04J08	-	-	-	-	+	-	+	-	-
Arthrobacter	SP003	-	++	-	-	+	-	+	+	-
Bacillus	AW25M04	-	-	-	-	+	-	+	+	-
Bacillus	GB04J31	-	-	-	-	-	+	-	-	-
Clavibacter	SP011	-	++	-	-	+	-	+	-	++
Clavibacter	SP033	-	++	-	-	+	-	+	-	++
Filibacter	KH04J17	-	-	-	-	-	-	-	-	-
Filibacter	AW28M30	-	-	-	-	+	-	-	-	-
Knoellia	SP073	-	-	++	-	-	++	-	++	++
Leifsonia	SP050	-	-	-	-	+	-	+	+	-
Leifsonia	AW28M06	-	-	-	-	+	-	+	-	-
Leifsonia	AW02J23	-	-	-	-	+	+	+	+	-
Microbacterium	AW28M07	-	+	-	-	+	-	+	-	-
Microbacterium	AW28M15	-	-	-	-	+	-	+	-	-
Microbacterium	SP006	-	++	-	-	+	-	-	-	-
Micrococcus	AW19M49	-	-	-	-	++	-	+	+	-
Micrococcus	SP063	-	-	-	-	-	-	++	++	-
Nesterenkonia	AW19M55	-	+	-	-	-	+	++	+	-
Nocardiopsis	BA19M08	-	-	-	-	-	-	+	+	-
Planococcus	AW02J18	-	-	-	-	++	-	+	+++	-
Planococcus	GB02J13	-	++	-	-	-	-	-	+	-
Plantibacter	AW25M38	-	-	-	-	++	-	-	-	-
Pseudomonas	AW19M56	-	+	-	-	-	-	-	++	-
Psycrobacillus	AW28M34	-	-	++	-	-	+	-	-	-
Rhodococcus	AW19M46	-	-	-	-	-	-	+	+	-
Rhodococcus	AW25M09	+	-	++	-	-	++	-	++	-
Rhodococcus	GB23J02	-	-	-	-	-	-	+	-	-
Rhodococcus	MP02J07	-	-	-	-	-	-	+	-	-
Rhodococcus	SP061	-	-	-	-	-	+	+	-	-
Rhodococcus	SP062	-	-	-	-	-	+	+	-	-
Salinibacterium	SP028	-	-	-	+	+	-	-	-	-
Sanguibacter	SP022	++	-	++	-	-	++	-	++	-
Staphylococcus	AW02J12	-	-	-	-	-	-	++	+	-
Staphylococcus	SP052	-	-	-	-	-	-	-	-	-
Streptomyces	AW19M35	-		-	-	-	-	-	-	-
Streptomyces	BA19M03	-	-	-	-	-	+	+	+	-
Streptomyces	AW19M42	-	-	++	-	-	++	-	++	++
Tomitella	SP012	-	-	-	-	-	+	+	-	-

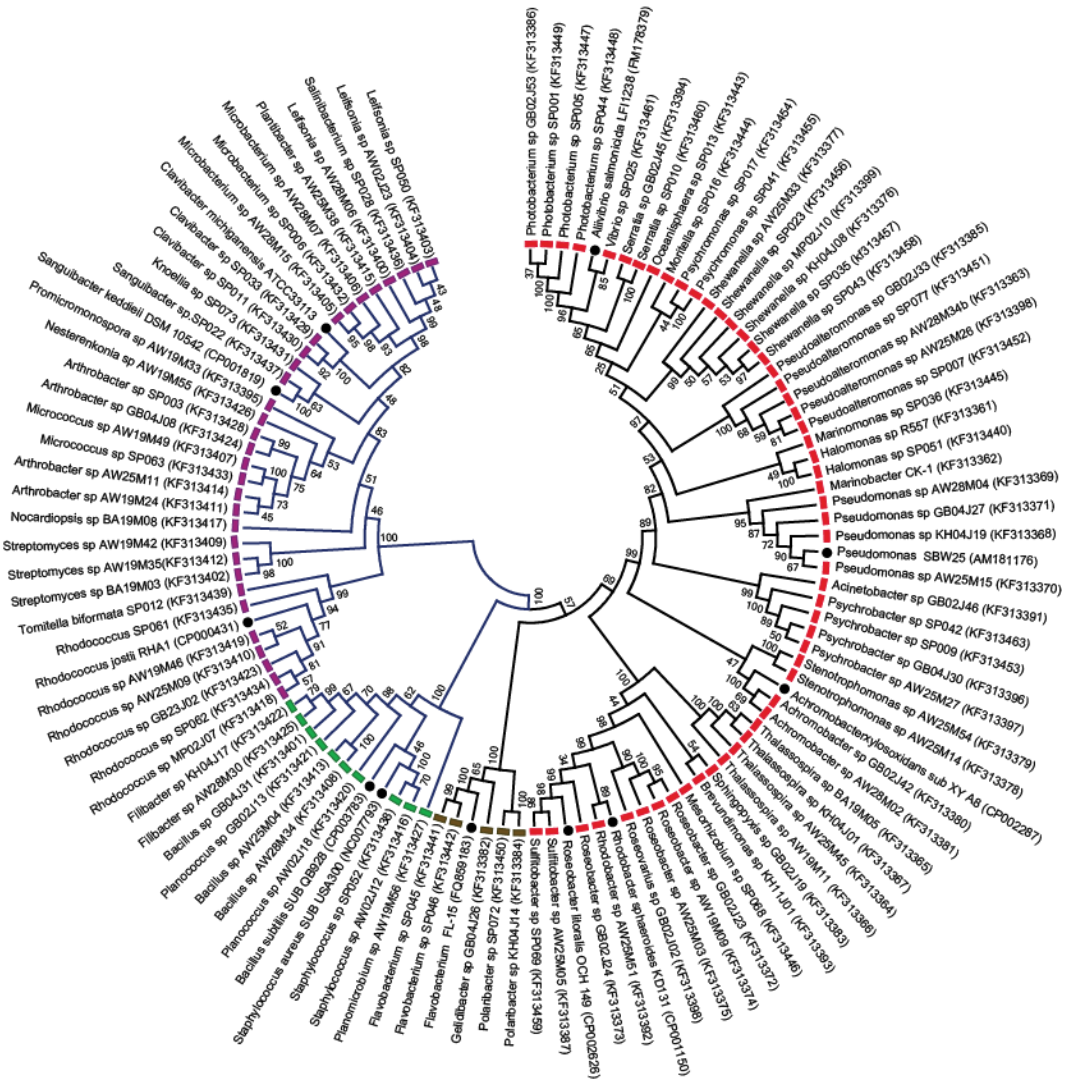
Table 4. Hydrolase activities of 60 Gram-negative marine bacterial isolates from Lofoten and Svalbard. Halo size is indicated by: (+) small halo (3 mm), (++) medium halo (5 mm), (+++) large halo (10 mm) and (-) no indication of halo.

	Alginate lyase		Amylase		Cellulase		Chitinase		DNase		Gelatinase		Lipase		Protease		Xylanase	
GRAM -	4°C	20°C	4°C	20°C	4°C	20°C	4°C	20°C	4°C	20°C	4°C	20°C	4°C	20°C	4°C	20°C	4°C	20°C
Achromobacter AW28M02	-		-		-		-		-		-		-		-		-	
Achromobacter GB02J42	-		-	+	-		-		+	++	+	+	-		-		-	
Acinetobacter GB02J46	-		-	+	-		+	++	-	++	+	++	+	++	-		-	
Brevundimonas KH11J01	-		-		-		-	+++	+	++	+	+++	+	+	++	++	-	
Flavobacterium SP045	-		+	++	-		-		-		-		-		+	-	-	
Flavobacterium SP046	-		+	++	-		-		+	+	-		-		++	-	-	
Gelidibacter GB04J26	-		-		-		-		-	++	++	++	-		++	+++	-	
Halomonas R5-57	-		-		-		-	+	-	+	-	+	+	-	-		-	
Halomonas SP051	-		-		-		-		+	+	-		-		-		-	
Marinobacter CK-1	+	++	-	+	-		-		+	++	+	++	+	++	++	++	-	
Marinomonas SP036	-		-		-		-		-	+	-		-	+	-		-	
Mesorhizobium SP068	-		-		-		-		-		-		-		-		-	
Moritella SP016	-		+	++	+	++	-		+	+	-		+	+	++	-	-	
Oceanisphaera SP013	-		-		+	++	-		-		-		-		-		-	
Photobacterium GB02J53	-		-		-		+	++	-		-		+	++	-		-	
Photobacterium SP001	-		-		-		+	++	-		-		-		-		-	
Photobacterium SP044	-		+	+	+	++	-		-	++	-		-	+	-	+	+	-
Photobacterium SP005	-		+	++	-		+	++	+	+	-		-		-		-	
Polaribacter KH04J14	-		+	+	-		-		-	++	++	++	+	+	++	++	-	
Polaribacter SP072	-		+	+	-		+	-	-	+	-		+	+	++	++	-	
Promiconospora AW19M33	-		-		-		-	++	-	+	-	+	-	+	-	+	+	+++
Pseudoalteromonas AW25M26	-		-		-		-		+	++	++	++	+	++	++	++	-	
Pseudoalteromonas AW28M34b	+	++	-	+	-		-		+	++	+	++	+	++	++	++	-	
Pseudoalteromonas GB02J33	-		++	+++	-		-		+	++	++	++	+	+	++	+++	-	
Pseudoalteromonas SP007	-		+	-	-		-		+	++	-		+	+	++	++	-	
Pseudoalteromonas SP077	-		+	++	-		+	+	+	++	-		+	++	-		-	
Pseudomonas AW28M04	-		-	+	-		-	++	-	+	+	+	+	+	-		-	
Pseudomonas GB04J27	-		-		-		-	++	-		-	++	+	+	+	+	-	
Pseudomonas AW25M15	-		-		-		-		-		++	-	+	++	+	++	-	
Pseudomonas KH04J19	-		-		-		-		-		++	-	+	+	+	++	-	

Table 4. Cont.

	Alginate lyase	Amylase		Cellulase		Chtinase		DNase		Gelatinase		Lipase		Protease		Xylanase	
GRAM -	4°C/20°C	4°C/20°C		4°C/20°C		4°C/20°C		4°C/20°C		4°C/20°C		4°C/20°C		4°C/20°C		4°C/20°C	
Psychrobacter AW25M27	-	+	++	-		-		+	++	+	++	+	+	++	++	-	
Psychrobacter GB04J30	-	-		-		-		-		-		+	+	-		-	
Psychrobacter SP009	-	+	++	-		+	-	-		-		+	+	-	++	-	
Psychrobacter SP042	-	+	++	-		-		+	+	-		+	++	+	++	-	
Psychromonas SP017	-	-		-		-		++	++	-		+	+	++	++	-	
Psychromonas SP041	-	-	+	+	++	+	-	-	++	-	+	+	++	-		-	
Rhodobacter AW25M51	-	-		-		-		+	++	-		+	+	++	++	-	
Roseobacter GB02J23	-	-		-		-	++	-		-	+	-		-	+	-	
Roseobacter GB02J24	-	-		-		-	++	-	+	-		+	+	-		-	
Roseobacter AW25M03	-	-		-		-	++	+	++	-	++	+	+	-		-	
Roseobacter AW19M09	-	-		-		-		-		-		-		-		-	
Roseovarius GB02J02	-	-	+	-		-		-	++	-		+	++	-		-	
Serratia GB02J45	-	+	++	-		-		-		+	++	+	+	++	++	-	
Shewanella KH04J08	-	-	+	-		-		+	+	-	++	++	+	++	+++	-	
Shewanella AW25M33	-	-		-		-		+	+	-	++	+	+	++	++	-	
Shewanella MP02J10	-	+	+	-		+	++	+	+	-		+	+	-	+	-	
Shewanella SP023	-	+	+	-		-		-		-		-		-		-	
Shewanella SP035	-	-		-	++	-		-	++	-		+	+	++	+	-	
Shewanella SP043	-	+	+	-		-		++	++	++	-	++	+	++	+	-	
Sulfitobacter SP069	-	+	+	-		+	+	-		-		+	+	-		-	
Serratia SP010	-	+	++	+	++	++	++	+	++	-		+	+	-		++	+++
Sphingopyxis GB02J19	-	-		-		-		-		-		-		-		-	
Stenotrophomonas AW25M54	-	-		-		+	++	+	++	++	++	+	+	++	++	-	
Stenotrophomonas AW25M14	-	+	+	-		-	+	+	++	-		+	+	++	++	-	
Sulfitobacter AW25M05	-	-		-		+	++	-		-		+	+	-		-	
Thalassospira AW2545	-	-		-		-		+	++	+	+	+	+	++	++	-	
Thalassospira AW19M11	-	-		-		-		+	++	-		-		-		-	
Thalassospira KH04J01	-	-		-		-		-		-		-		-		-	
Thalassospira BA19M05	-	-		-		-		-	+	-		-		-		-	
Vibrio SP025	-	++	+	-		-		+	++	-		+	+	-	+	+	-

Figure 1


Photobacterium sp GB02J53 (KF313386)
Photobacterium sp SP001 (KF313449)
Photobacterium sp SP005 (KF313447)
Photobacterium sp SP044 (KF313448)
Aliivibrio salmonicida LFI1238 (FM178379)
Vibrio sp SP025 (KF313461)
Serratia sp GB02J45 (KF313394)
Oceanisphaera sp SP010 (KF313460)
Moritella sp SP016 (KF313443)
Psychromonas sp SP013 (KF313444)
Psychromonas sp SP017 (KF313454)
Shewanella sp AW25M33 (KF313455)
Shewanella sp SP023 (KF313456)
Shewanella sp MP02J10 (KF313377)
Shewanella sp KH04J08 (KF313399)
Shewanella sp SP035 (KF313376)
Shewanella sp SP043 (KF313467)
Pseudoalteromonas sp GB02J33 (KF313456)
Pseudoalteromonas sp SP077 (KF313385)
Pseudoalteromonas sp AW28M34b (KF313451)
Pseudoalteromonas sp AW25M26 (KF313363)
Pseudoalteromonas sp SP007 (KF313398)
Pseudoalteromonas sp SP036 (KF313452)
Marinomonas sp R557 (KF313445)
Halomonas sp SP051 (KF313361)
Halomonas sp KH04J19 (KF313440)
Marinobacter CK-1 (KF313362)
Pseudomonas sp AW28M04 (KF313369)
Pseudomonas sp GB04J27 (KF313371)
Pseudomonas sp KH04J19 (KF313368)
Pseudomonas SBW25 (AM181176)
Pseudomonas sp AW25M15 (KF313370)
Acinetobacter sp GB02J46 (KF313391)
Psychrobacter sp SP042 (KF313463)
Psychrobacter sp SP009 (KF313453)
Psychrobacter sp GB04J30 (KF313396)
Psychrobacter sp AW25M27 (KF313397)
Stenotrophomonas sp AW25M54 (KF313379)
Achromobacterxylosoxidans sub XY A8 (CP002287)
Achromobacter sp GB02J42 (KF313390)
Achromobacter sp AW28M02 (KF313381)
Thalassospira sp BA19M05 (KF313365)
Thalassospira sp KH04J01 (KF313387)
Thalassospira sp AW25M45 (KF313364)
Thalassospira sp AW19M11 (KF313366)
Sphingopyxis sp GB02J19 (KF313395)
Sphingopyxis sp SP068 (KF313446)
Brevundimonas sp KH11J01 (KF313372)
Mesorhizobium sp GB02J23 (KF313374)
Roseobacter sp AW19M09 (KF313375)
Roseobacter sp AW25M03 (KF313368)
Roseovarius sp GB02J02 (KF313388)
Rhodobacter sphaeroides KD131 (CP001150)
Rhodobacter sp AW28M51 (KF313392)
Rhodobacter sp GB02J24 (KF313373)
Roseobacter litoralis OCH 149 (CP002625)
Roseobacter sp AW25M05 (KF313387)
Silicibacter sp SP069 (KF313459)
Polaribacter sp KH04J14 (KF313384)
Polaribacter sp SP072 (KF313450)
Gelidibacter sp GB04J26 (KF313382)
Gelidibacter FL-15 (FQ859183)
Flavobacterium sp SP046 (KF313441)
Flavobacterium sp SP045 (KF313427)
Flavobacterium sp AW19M56 (KF313416)
Flavobacterium sp AW02J12 (KF313438)
Planomicrobium sp SP052 (KF313442)
Staphylococcus sp AW02J18 (KF313420)
Staphylococcus aureus SUB USA300 (NC007793)
Staphylococcus subtilis SUB QB928 (CP003719)
Bacillus sp AW28M34 (KF313408)
Bacillus sp AW25M04 (KF313413)
Bacillus sp GB02J13 (KF313401)
Bacillus sp GB04J31 (KF313421)
Filibacter sp AW28M30 (KF313422)
Filibacter sp KH04J17 (KF313425)
Rhodococcus sp MP02J07 (KF313418)
Rhodococcus sp SP062 (KF313434)
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Rhodococcus sp AW19M46 (KF313419)
Rhodococcus josti RHA1 (CP000431)
Tomitella biformata SP012 (KF313435)
Rhodococcus SP061 (KF313431)
Streptomyces sp BA19M03 (KF313402)
Streptomyces sp AW19M35(KF313412)
Streptomyces sp AW19M42 (KF313409)
Nocardiopsis sp BA19M08 (KF313417)
Arthrobacter sp AW19M24 (KF313411)
Arthrobacter sp AW25M11 (KF313414)
Micrococcus sp SP063 (KF313433)
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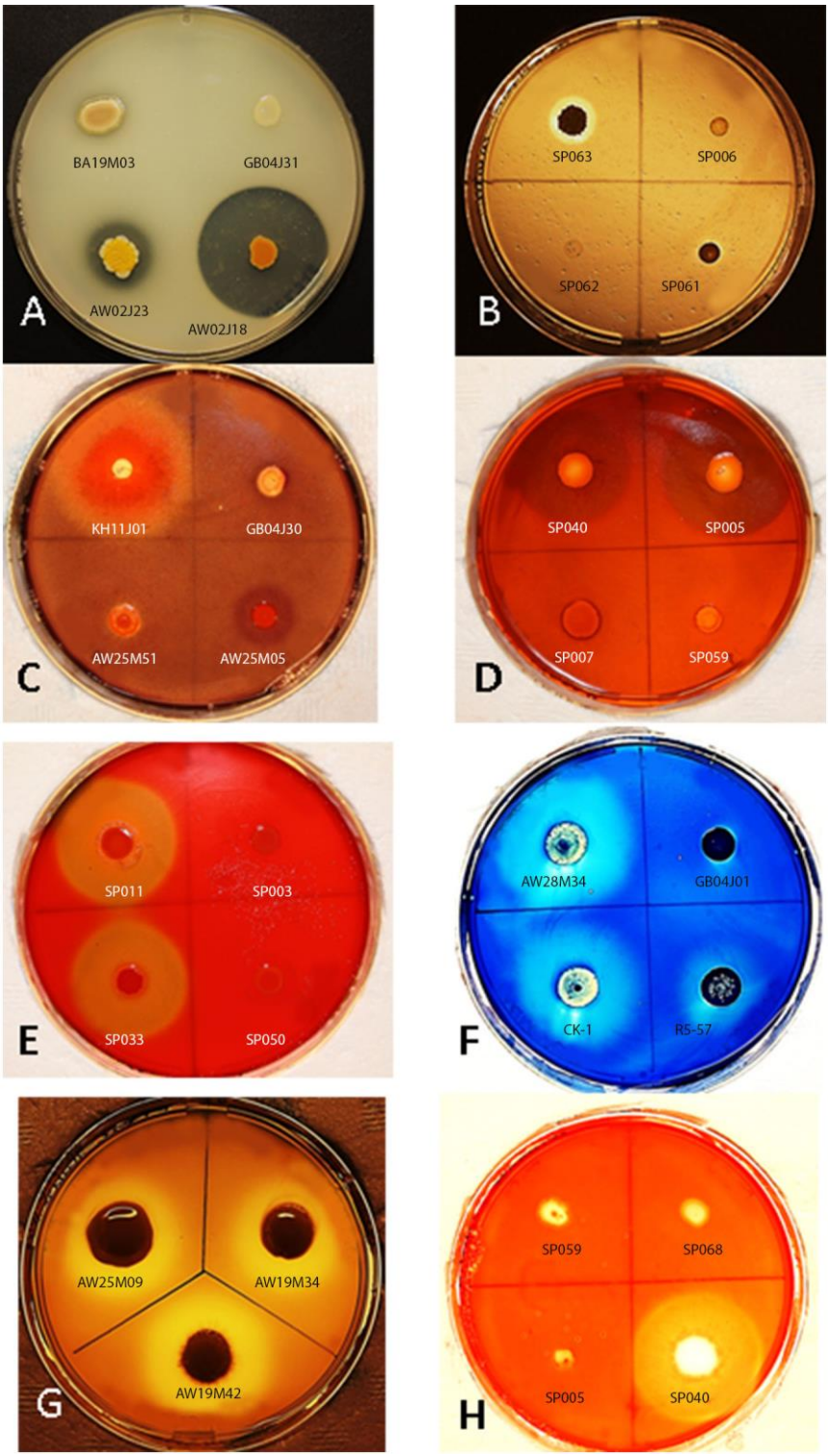
Arthrobacter sp GB04J08 (KF313424)
Arthrobacter sp SP003 (KF313426)
Arthrobacter sp AW19M55 (KF313395)
Nesterenkonia sp AW19M33 (KF313396)
Promicromonospora sp AW19M33 (CP001819)
Sanguibacter keddieii DSM 10542 (CP001819)
Sanguibacter sp.SP022 (KF313437)
Knoellia sp SP073 (KF313430)
Clavibacter sp SP011 (KF313429)
Clavibacter sp SP033 (KF313431)
Clavibacter michiganensis ATCC33113
Microbacterium sp AW28M15 (KF313430)
Microbacterium sp SP006 (KF313409)
Microbacterium AW28M07 (KF313406)
Planlibacter sp AW25M38 (KF313415)
Leifsonia sp AW02J23 (KF313404)
Salinibacterium sp SP028 (KF313440)
Leifsonia sp SP050 (KF313403)

Figure 2


BA19M03
GB04J31
AW02J23
AW02J18
A
SP063
SP006
SP062
SP061
B
KH11J01
GB04J30
AW25M51
AW25M05
C
SP040
SP005
SP007
SP059
D
SP011
SP003
SP033
SP050
E
AW28M34
GB04J01
CK-1
R5-57
F
AW25M09
AW19M34
AW19M42
G
SP059
SP068
SP005
SP040
H