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Research Letter

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Profiling the microbial community of a Triassic halite deposit in Northern Ireland: an environment with significant potential for biodiscovery

Julianne Megaw, Stephen A. Kelly, Thomas P. Thompson, Timofey Skvortsov and Brendan F. Gilmore*

Biofilm Research Group, School of Pharmacy, Queen's University Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, UK

*Corresponding author: School of Pharmacy, Queen's University Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, UK. Tel: +028 9097 2086; E-mail: b.gilmore@qub.ac.uk

One sentence summary: The microbial community of Kilroot salt mine (a Triassic halite deposit in Northern Ireland) was profiled, and appears to represent an excellent resource with high potential for biodiscovery.

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ABSTRACT

Kilroot salt mine, a Triassic halite deposit located in County Antrim, Northern Ireland, is the only permanent hypersaline environment on the island of Ireland. In this study, the microbiome of this unstudied environment was profiled for the first time using conventional and enhanced culturing techniques, and culture independent metagenomic approaches. Using both conventional isolation plates and iChip devices, 89 halophilic archaeal isolates from six known genera, and 55 halophilic or halotolerant bacterial isolates from 18 genera were obtained, based on 16S rRNA gene sequencing. The archaeal isolates were similar to those previously isolated from other ancient halite deposits, and as expected, numerous genera were identified in the metagenome which were not represented among the culturable isolates. Preliminary screening of a selection of isolates from this environment identified antimicrobial activities against a panel of clinically important bacterial pathogens from 15 of the bacterial isolates and one of the archaea. This, alongside previous studies reporting the discovery of novel biocatalysts from the Kilroot mine microbiome, suggests that this environment may be a new, untapped source of of chemical diversity with high biodiscovery potential.

Keywords: Halophiles; haloarchaea; halite; isolation; metagenome; biodiscovery

INTRODUCTION

Hypersaline waters with salt concentrations up to and beyond the point of saturation contain dense populations of halophilic microorganisms, with the three domans of life all represented (Gunde-Cimerman, Plemenitaš and Oren 2018). High population densities are enabled in these environments as they tend to be nutrient-rich and located in warm climates, but because of the selective pressures of the high salinity and additional challenges (temperature extremes, low dissolved oxygen, UV radiation and high pH), they tend to be of low diversity (Ventosa and Arahal 2002; Ma *et al.* 2010; Jaakkola *et al.* 2016b).

Evaporite deposits represent the desiccated remains of ancient hypersaline waters. During the Earth's history, extensive sedimentation of halite (NaCl) and other minerals from salt-saturated seas or lakes occurred, with an estimated 1.3 million km^3 of halite deposited during the late Permian and early Triassic periods alone (Fendrihan *et al.* 2006). Microorganisms

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present in these environments became trapped within fluid inclusions formed during salt crystallization (Norton and Grant 1988), with data from previous studies suggesting that some of these have remained viable within the halite ever since its deposition, with viable archaea and bacteria having been recovered from within samples dating as far back as the Permian period (Norton, McGenity and Grant 1993; Stan-Lotter *et al.* 1993; Vreeland, Rosenzweig and Powers 2000; Gruber *et al.* 2004; Jaakkola *et al.* 2014). When these deposits were formed, the continental land masses took the form of the supercontinent Pangaea which, driven by plate tectonics, began to fragment and displace the continents (and the halite deposits) about 100 mya, with the largest deposits transported to northern regions such as Siberia, Northern and Central Europe, and the midcontinent basin in North America (Fendrihan *et al.* 2006).

Salt mines are one of the few means of accessing deeply buried halite deposits to obtain samples for study. One previously unstudied deposit is Kilroot salt mine, located approximately 3 km northeast of Carrickfergus, Co Antrim, Northern Ireland. Part of the Triassic Mercia Mudstone Group and composed primarily of bedded halite, gypsum and siliciclastic mudstones (Andeskie *et al.* 2018), this deposit is the only source of rock salt in Ireland. It has been mined by the Irish Salt Mining and Exploration Co. since 1965, with current operations occurring at a depth of approximately 300 m. At present, the mine produces 400–500 000 tonnes of crushed rock salt annually for de-icing roads during the winter period. Pulverized fuel ash (PFA) from the adjacent coal-fired power station is mixed into the concrete used for backfilling in the mine following excavation.

The aim of this study was to profile the microbiome of Kilroot mine, using conventional culture-dependent approaches (including augmented culturing techniques) and culture-independent approaches. Previously, researchers have successfully extracted DNA from hypersaline samples and identified the resident microorganisms via probing with universal primers or metagenomics (Radax, Gruber and Stan-Lotter 2001). Numerous recent studies have given insight into the diversity of uncultured microbiota in hypersaline water bodies (summarized in (Ventosa *et al.* 2015)), but there are fewer metagenomic studies of hypersaline environments dominated by crystalline salt, and to our knowledge none on metagenomic profiling of material derived from Triassic halite. With the growing understanding of the potential biotechnological applications of extremophiles and their products (Coker 2016), the purpose of this study, in addition to examining the culturable and non-culturable microbiota present within this environment, was to mine its potential as an untapped source of chemical diversity for future applications.

MATERIALS AND METHODS

Sample collection

Halite fragments were collected from Kilroot mine (54.7331°N, 5.7509°W) (Fig. 1) at a depth of 300 m, from a working rock face which was newly exposed by recent blasting. The site has a constant temperature of 15°C. Brine samples were collected in sterile bottles from a small saturated brine stream in a lower part of the mine, formed by percolating groundwater. Halite samples were also taken for incorporation into isolation and culture media. A sample of brine-saturated PFA sediment was also collected.



Figure 1. Photographs of (clockwise from top left) the interior of Kilroot mine, showing tunnels and salt processing machinery, the freshly blasted rock face from which halite samples were obtained, halite samples showing characteristic red/pink pigmentation, and stream from which brine samples were obtained.

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Isolation using conventional methods

Large halite fragments were broken and pieces from the inside were used (to reduce the chances of any isolates obtained being recent surface contaminants); these were surface-sterilized using the protocol described in Fish *et al.* (2002). These were placed in sterile 50 mL centrifuge tubes and dissolved by gradual addition of sterile distilled water, to minimise the osmotic shock experienced by any microorganisms present within the halite. PFA samples were treated as described in Thaker, Waglechner and Wright (2014) for the isolation of actinomycetes. 100 μL of the dissolved halite, undiluted brine sample or PFA was spread on the surface of plates of solid isolation media, the compositions of which are shown in Table S1 (Supporting Information). Four plates of each medium were prepared for each sample, with each plate incubated under different conditions (28, 37 (aerobic and anaerobic) and 45°C). Anaerobic isolation was conducted using a modular atmospheric controlled system anaerobic workstation (DW Scientific, UK). Plates were incubated for up to eight weeks in sealed bags to minimize evaporation and were periodically checked for growth. Any observed colonies were streaked onto fresh plates of the same medium and re-streaked to ensure culture purity.

Isolation using the iChip

Inoculation of iChip devices followed the protocol in Nichols *et al.* (2010) with some modifications. PFA was held at the bottom of the device and was also used as the inoculum. A 100 g PFA sample was subjected to pre-treatments known to facilitate the isolation of actinomycetes: heat treatment at 50°C for 60 min, followed by the addition of 5% (w/v) SDS and 1.4% (w/v) phenol. Sterile water was added to make a slurry from the sample, which was then used to inoculate the iChip devices. Agar plugs were created using actinomycete isolation agar with either 10 or 20% (w/v) halite added. Each device was incubated at 37°C for 4 weeks. Following this period, agar plugs were observed for growth and selected plugs were picked and streaked onto fresh plates, which were incubated for a further 4 weeks.

DNA extraction and 16S rRNA gene sequencing

For bacterial isolates, total DNA was extracted using the GenElute Bacterial Genomic DNA kit (Sigma-Aldrich, UK), as per the manufacturer's instructions. Instead of using the included lysis solutions, haloarchaeal cells were lysed by the addition of nuclease-free water, after which the procedure was continued as per the manufacturer's instructions. 16S rRNA genes were amplified using the universal primer pairs 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) for bacterial isolates, and Arch21F (5′-TTCCGGTTGATCCYGCCGGA-3′) and Arch958R (5′-YCCGGCGTTGAMTCCAATT-3′) for archaeal isolates, with Sanger sequencing of the PCR products conducted by Eurofins Genomics (UK), using the 27F and Arch21F primers. Sequence data were analyzed by comparison with the sequences available within the National Centre for Biotechnology Information (NCBI) GenBank database.

Phylogenetic reconstruction

Evolutionary analyses were conducted in MEGA7 (Kumar, Stecher and Tamura 2016) on partial 16S rRNA gene sequences of the isolates. A total of 55 bacterial isolate sequences were aligned with 33 bacterial type strain sequences, and 89 archaeal isolate sequences were aligned with 20 archaeal type strain sequences using the built-in automated alignment software Clustal W. Alignments were trimmed at their 5′ and 3′ ends to yield sequences of the same length. The evolutionary histories of the two groups were inferred using the Neighbor-joining method (Saitou and Nei 1987), and bootstrap consensus trees were inferred from 1000 permutations of the data sets (Felsenstein 1985). The type strains *Thermococcus gammatolerans* DSM 15229 and *Aquifex pyrophilus* Kol5a were used as outgroups for the archaeal and bacterial trees, respectively. The evolutionary distances were computed using the Jukes-Cantor method (Jukes and Cantor 1969), with pair-wise deletion for gap treatment, and are in the units of the number of base substitutions per site. All ambiguous positions were removed for each sequence pair. There were a total of 507 positions in the final archaeal data set and 674 positions in the final bacterial data set.

DNA extraction and metagenomic sequencing

A two-litre brine sample was vacuum filtered through a 0.2 μm nitrocellulose filter membrane, which was then cut into several pieces and DNA extracted using GenElute™ Bacterial Genomic DNA Extraction kit (Sigma-Aldrich, UK) as per the manufacturer's instructions. Extracted DNA was sent to MR DNA (Shallowater, TX, USA) for library preparation (Nextera XT) and metagenomic sequencing on the Illumina MiSeq platform. The resulting metagenomic dataset comprised 7.63 M 250 bp-long paired-end reads. Initial quality control was performed with FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and reads were processed with BBMap v 33.54 (http://sourceforge.net/projects/bbmap/). Briefly, all reads with an average Q-score < 10 or containing Ns were discarded, the reads were then trimmed of adapters and quality-trimmed. The assembly of the processed reads into contigs was performed with SPAdes genome assembler v 3.10.1 (Bankevich *et al.* 2012) in meta mode with default parameters. Microbial community structure analysis was conducted using both RefSeq annotation in MG-RAST (Meyer *et al.* 2008) and Kraken v2.0.6-beta (Wood and Salzberg 2014), followed by visualization with Pavian v0.8.2 (Breitwieser and Salzberg 2016).

Screening for antimicrobial activity

A selection of bacterial and archaeal isolates were grown as liquid cultures in their optimal medium at their optimal temperatures (data not shown) until they reached stationary phase. The contents of each flask were extracted overnight in three volumes of ethyl acetate (EtOAc). The organic (top) phase was retained and concentrated by rotary evaporation, weighed, and redissolved in EtOAc to obtain stock solutions of each extract with a concentration of 50 mg mL^{-1}. Extractions of the culture media were also conducted as a control. Extracts were then screened against a panel of clinically relevant bacterial pathogens using the Kirby-Bauer disk diffusion method (Bauer *et al.* 1966), using 10 μL (0.5 mg) extract per disk. Metagenomic sequence data were also screened for the presence of antimicrobial biosynthetic genes using BAGEL3 (van Heel *et al.* 2013) and AntiSMASH 4.0 (Blin *et al.* 2017).

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RESULTS

Culturable and non-culturable microbiome analysis

A total of 89 archaeal and 55 bacterial isolates were obtained from the Kilroot mine samples. With the exception of 24 archaeal isolates which came from the PFA, all other isolates were obtained from brine, and none from the surface-sterilized halite. Partial 16S sequences (Table S2, Supporting Information) were compared with those contained within the NCBI database to identify the isolates based on the closest match at either the genus or species level; these are presented in Tables S3 and S4 (Supporting Information), with phylogenetic relationships shown in Figs 2 and 3. The closest matches of the majority of the isolates were known halophilic genera or species. Sequence analysis of the archaeal isolates indicated that they belonged to six named genera: *Halorubrum* (77.5%), *Halococcus* (12.4%), *Haloarcula* (3.4%), *Halohasta* (3.4%), *Halobacterium* (2.2%), and *Haloferax* (1.1%) (Fig. 4A), with at least 12 identified in the metagenome analysed by MG-RAST (Fig. 4C), and at least 10 with Kraken analysis (Fig. 5). 18 bacterial genera were represented among the isolates (Fig. 4B) with 29 identified by MG-RAST (Fig. 4D). Metagenomic profiling performed with Kraken classified only 28.44% of all reads. The

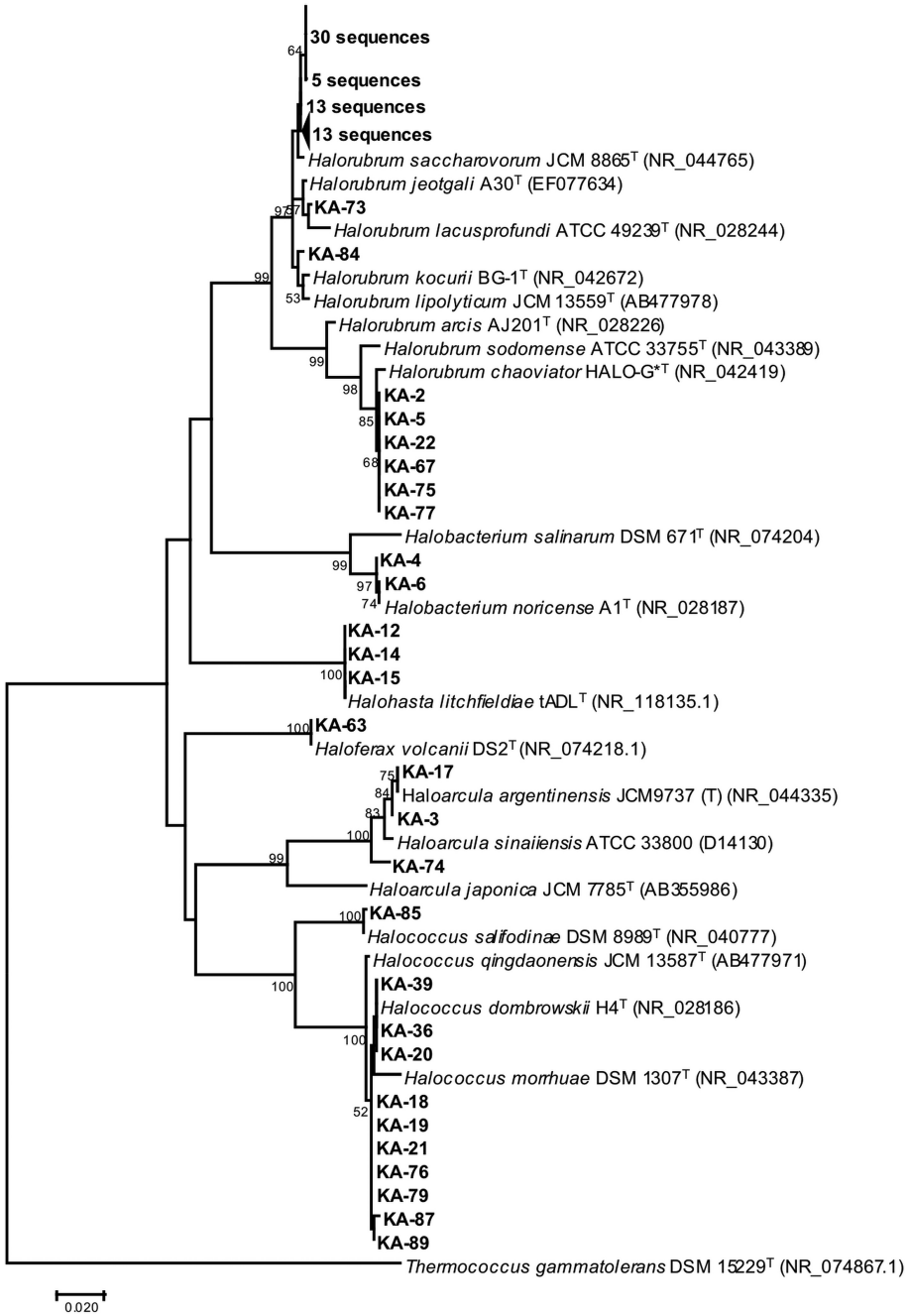


Figure 2. Evolutionary relationships of halophilic archaea isolated from Kilroot salt mine. The isolates obtained during this study are presented in bold, and all reference strains are presented with their GenBank accession number. The phylogenetic tree, based on 16S rRNA gene sequences, was constructed by the Neighbor Joining method. Bootstrap values are based on 1000 replicates and are shown for branches with greater than 50% support. The bar represents 2% base substitutions.

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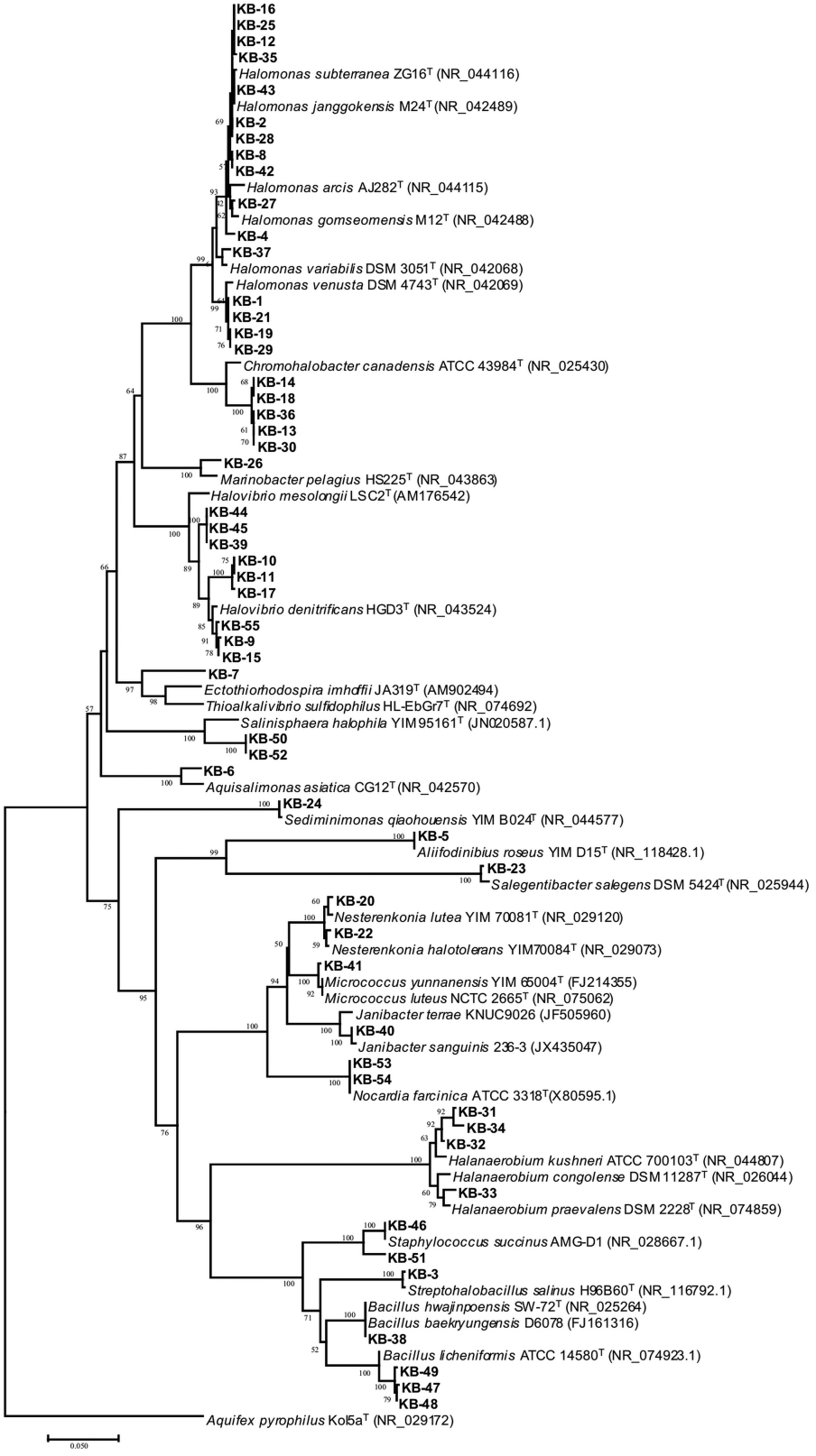


Figure 3. Evolutionary relationships of halophilic and halotolerant bacteria isolated from Kilroot salt mine. The isolates obtained during this study are presented in bold, and all reference strains are presented with their GenBank accession number. The phylogenetic tree, based on 16S rRNA gene sequences, was constructed by the Neighbour Joining method. Bootstrap values are based on 1000 replicates and are shown for branches with greater than 50% support. The bar represents 5% base substitutions.

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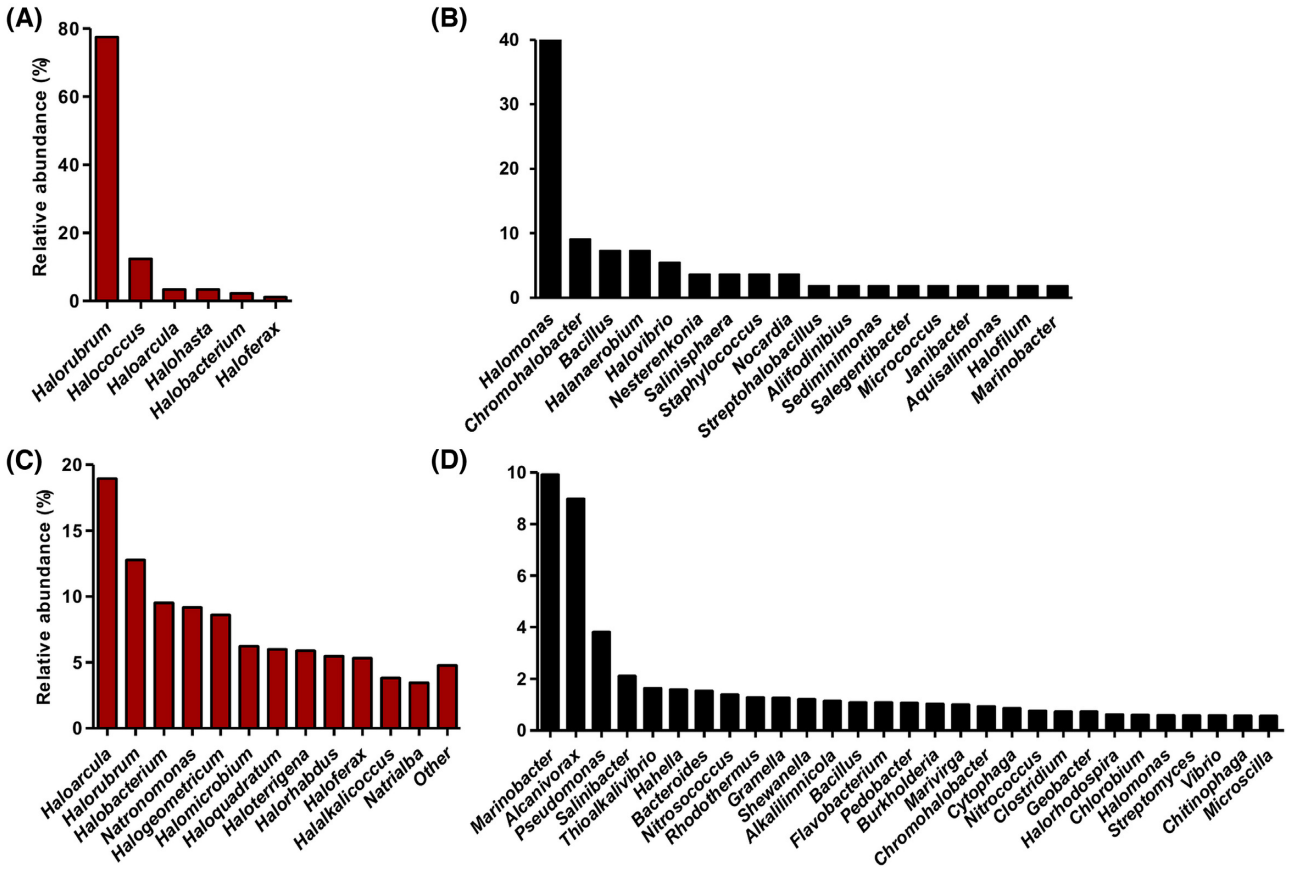


Figure 4. Relative abundance of different genera of archaeal **(A)**, and bacterial **(B)**, isolates from a Kilroot salt mine brine sample, in comparison to archaeal **(C)**, and bacterial **(D)**, genera identified from the same sample by the refSeq annotation function in MG-RAST.

majority of classified reads originated from archaea (71.73%), while bacterial reads constituted 26.65%, and the remaining reads were of viral and fungal origin. Six major archaeal genera in terms of the number of classified reads were *Halorubrum* (11.69%), *Halobacterium* (10.98%), *Haloarcula* (7.48%), *Haloferax* (3.22%), *Natrinema* (3.02%) and *Halopenitus* (2.84%). Among bacteria, *Pseudomonas*, *Streptomyces* and *Marinobacter* were identified as three major genera, accounting for 2.56%, 1.70% and 1.11% of all classified reads, respectively.

Antimicrobial activity of culture extracts

Based on screens using the Kirby-Bauer disk diffusion method, 16 of the isolates had antimicrobial activity against at least one, and up to six, of the 10 bacterial pathogens in the panel (Table 1). 15 of those showing activity were bacteria, but interestingly, one of the archaeal isolates (KA-7, identified as a strain of *Halorubrum saccharovorum*), had slight antimicrobial activity against *Pseudomonas aeruginosa* PAO1.

DISCUSSION

All the isolates obtained during this study were obtained from brine and PFA samples, with none from surface-sterilized halite. The success of other researchers in isolation of microorganisms from halite has been highly variable, with 1–2 viable isolates kg^{-1} reported from Permian and Triassic halite from British salt mines (Norton, McGenity and Grant 1993), up to 1.3×10^5 viable cells kg^{-1} in a Permian deposit at Bad Ischl, Austria (Stan-Lotter *et al.* 2000), and 1.0×10^4 cfu g^{-1} in the Permian Salado formation in New Mexico, USA (Vreeland *et al.* 1998). Archaeal diversity in ancient halite is known to be low, with few genera identified using conventional isolation or metagenomics (Jaakkola *et al.* 2016b). The Kilroot mine isolates also showed low diversity, and high similarity to those from previous studies, with four of the six genera (*Haloarcula*, *Halorubrum*, *Halobacterium*, and *Halococcus*) already identified in other ancient halites (Norton, McGenity and Grant 1993; Stan-Lotter *et al.* 2002; Stan-Lotter, Gruber and Radax 2002; Jaakkola *et al.* 2016b). In addition to these, we also isolated a representative of the genus *Haloferax*, and three from the more recently described genus *Halohasta* (Mou *et al.* 2012).

While the isolates we obtained were not derived directly from halite samples, they were predominantly isolated from saturated brine formed by water pooling on its surface deep within in the mine. This may not make it possible to eliminate all doubt that they were associated with the original deposit, although it seems unlikely that they could be from another source. In some locations it may be possible to identify the same species in surface and subsurface environments at multiple levels (Gramain *et al.* 2011), making it difficult to determine the precise origin of an isolate. However, Ireland does not have surface environments which would support extreme halophiles long-term, and taking into consideration the depth from which the samples were obtained, this suggests that these haloarchaea were associated with the original deposit, and their presence in the brine samples was because of the liberation of viable microorganisms from the halite when it was dissolved. Over the past 50 years, numerous reports have claimed long-term

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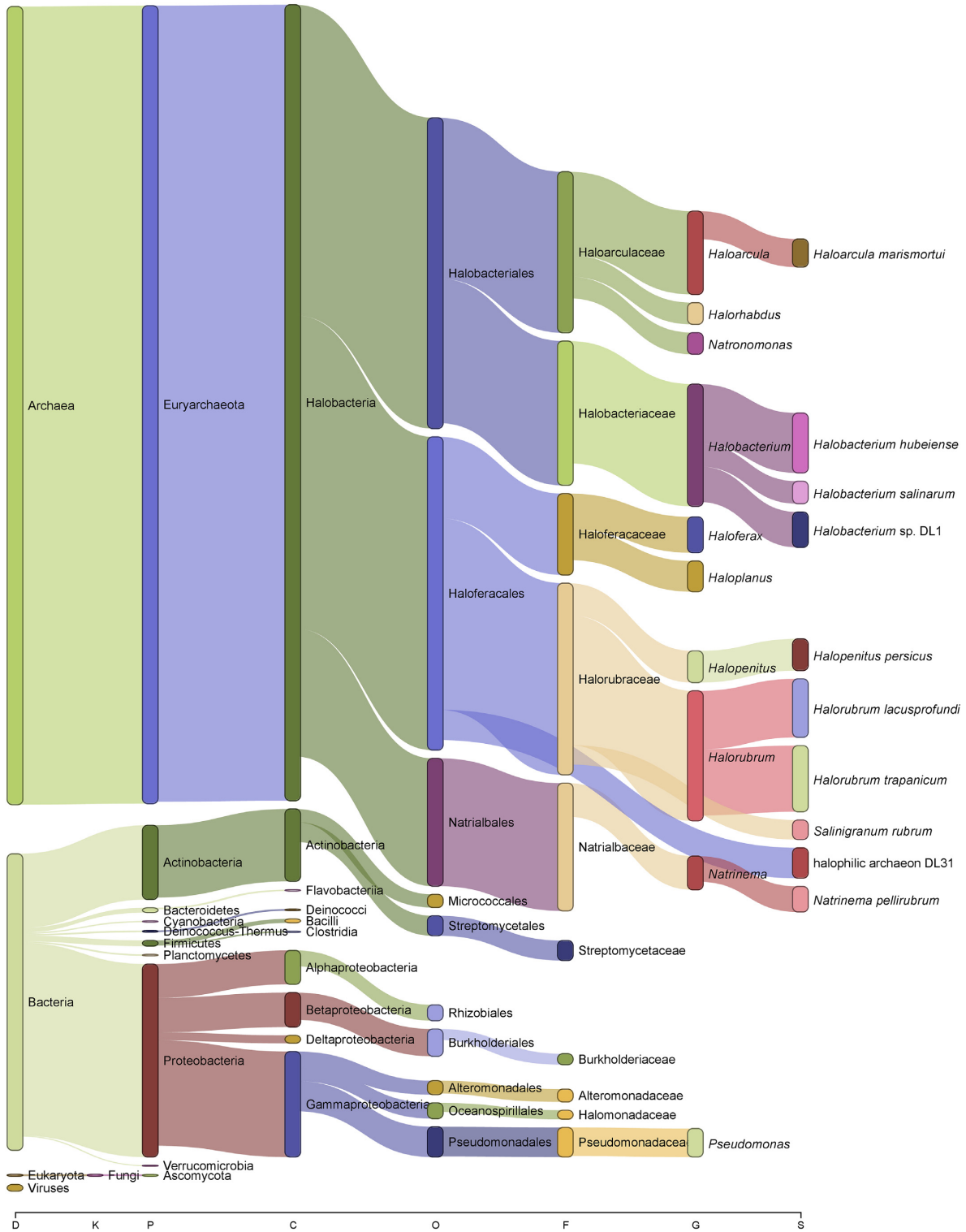


Figure 5. Microbial community structure of a brine sample from Kilroot salt mine, as determined by annotation in Kraken, followed by visualization with Pavian. Classified reads are presented at eight taxonomic levels, with the number of reads assigned to each taxon being proportional to their height in the Sankey diagram.

survival of microorganisms within halite deposits (summarised in Lowenstein, Schubert and Timofeeff 2011), and have shown that viable, similar haloarchaeal species have been consistently isolated from geographically separate halite samples by several groups around the world (Stan-Lotter *et al.* 1999; Legat *et al.* 2013). Identical species have also been isolated on separate occasions from samples obtained from the same site, several years apart (Stan-Lotter *et al.* 2002). In particular, the many well-described adaptations of *Halobacterium* species which enable prolonged survival inside halite, and the identification of similar *Halobacterium* isolates and sequences in this study, and numerous other studies of ancient halite samples (Gruber *et al.* 2004; Gramain

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Table 1. Antimicrobial activities of halophilic bacterial and archaeal isolates, as determined by disc diffusion assays. Numbers indicate diameter of zone of inhibition in mm; - indicates no inhibition was observed.

									Methicillin-resistant			
	Pseudomonas aeruginosa PAO1	*Escherichia coli* NCTC 12241	*Klebsiella pneumoniae* NCTC 9633	*Proteus mirabilis* ATCC 7002	*Staphylococcus aureus* NCTC 12981	Methicillin-resistant *Staphylococcus aureus* NCTC 10442	*Staphylococcus epidermidis* NCTC 13360	*Staphylococcus epidermidis* NCTC 11964	*Enterococcus faecalis* NCTC 775	*Enterococcus faecium* NCTC 7171
KA-7	7	-	-	-	-	-	-	-	-	-
KB-2	-	-	-	-	-	-	10	17	-	10.5
KB-4	-	-	-	-	12	11	15.5	18	8	14
KB-8	-	-	-	-	-	-	10.5	19.5	-	-
KB-12	-	-	-	-	8.5	8	-	22	-	-
KB-13	-	-	-	-	13	10	-	18	-	-
KB-14	-	-	-	-	12	10	-	-	-	-
KB-18	-	-	-	-	12	-	-	-	-	-
KB-19	-	-	-	-	9	10.5	9	25	-	-
KB-21	-	-	-	-	10.5	11.5	17.5	22	-	12.5
KB-25	-	-	-	12	-	-	-	12	-	-
KB-28	-	-	-	-	8	8	8.5	15.5	-	-
KB-31	-	7	-	-	9.5	9	-	-	-	-
KB-32	-	-	-	-	8	9	-	-	-	-
KB-33	-	-	-	-	-	-	9.5	-	-	-
KB-34	-	6.5	7	-	9	8.5	-	10	-	-

et al. 2011; Jaakkola *et al.* 2016a), supports the hypothesis that, in many cases, many of the isolates from subterranean halite deposits likely represent stable, preserved populations, and are not recent surface contaminants.

While many of the bacterial isolates represent known halophilic or halotolerant species, it would not be possible to say with the same level of confidence that they were also originally associated with the deposit. While bacteria have been isolated from ancient halite (Jaakkola *et al.* 2016b), brines from other salt mines have been dominated by haloarchaea (Norton, McGenity and Grant 1993; Payler *et al.* 2019). The bacterial isolates were mostly Gram-negative rods, particularly from the family *Halomonadaceae*, which contains the large genera *Halomonas*, *Halovibrio,* and *Chromohalobacter*, which were well represented among the isolates. We also isolated some less common species, including two representatives of the genus *Salinisphaera*, previously isolated from diverse saline and hypersaline environments including surface seawater (Bae *et al.* 2010), deep-sea hydrothermal vents (Crespo-Medina *et al.* 2009), a solar saltern (Park *et al.* 2012), brine from a salt well (Zhang *et al.* 2012b), and the surface of a deep-sea fish (Shimane *et al.* 2013), but never previously from a salt mine, to our knowledge. Four isolates (KB-31–34) were obligate anaerobes. Many halophiles benefit from anaerobic metabolism, as oxygen is poorly soluble in hypersaline waters, and many other saline environments have reduced O_2 levels, such as deep sediments, the lower layers of microbial mat communities (Ollivier *et al.* 1994), and fluid inclusions within halite crystals, where the oxygen is quickly depleted (McGenity *et al.* 2000).

As expected, both types of metagenomic analysis identified more genera than were isolated, as it is well understood that not all of the microbial species present in most environments can be successfully cultivated using standard methods (Staley and Konopka 1985; Lloyd *et al.* 2018). Discrepancies between the proportions of major genera among the isolates and metagenomic profiles can most likely be attributed to the culturing methods employed, which may have favored some taxonomic groups over others. Kraken analysis classified only 28.44% of reads, suggesting that this salt mine is still a reservoir of uncharacterised genetic diversity.

Enhanced culturing techniques are becoming increasingly popular, with the aim of isolating a greater proportion of the species present in environmental samples. IChip devices were set up with the primary objective of isolating actinomycetes. Saline or hypersaline environments are a potential source of these organisms (Jose and Jebakumar 2014; Ballav *et al.* 2015) which are known for for their bioactive secondary metabolites, but which are also difficult to isolate because of isolation plates becoming dominated by other, faster-growing species. PFA provided a suitable consistency for use in the iChip – similar to that of soil or other terrestrial sediments. This method has been used with great success for the isolation of novel and 'uncultivable' microbes (Nichols *et al.* 2010; Ling *et al.* 2015). We did not obtain any actinomycetes using this method, however, instead isolating numerous additional *Halorubrum* spp. Two actinomycetes of the genus *Nocardia* (KB-53, KB-54; Table S4, Supporting Information) were obtained on conventional isolation plates, however. Failure to isolate actinomycetes using the iChip has been noted previously; Nichols *et al.* (2010) suggested that *in situ* conditions may reduce their growth rate in comparison to *in vitro* conditions, necessitating considerably longer incubation times. This approach may therefore still be successful following further optimization of the pre-treatments and incubation period.

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Preliminary screens of culture extracts showed some promising inhibitory activities against a number of bacterial pathogens, with early results appearing to suggest that this environment may provide a novel, exploitable source of antimicrobial compounds. The activity of a *Hrr. saccharovorum* isolate against *Pseudomonas aeruginosa* was of particular interest. While haloarchaea are known to produce proteinaeous antimicrobial compounds (halocins), the majority of published studies have focused on their activity against closely related species (Besse *et al.* 2015). However, a limited number of studies have shown the possibility of cross-domain antimicrobial activity (Kavitha *et al.* 2011; Atanasova *et al.* 2013). As is frequently reported, new antibiotics are required as a matter of global urgency. In the early days of antibiotic discovery, new compounds were discovered using the 'Waksman platform', a systematic process of screening isolates against target pathogens, which was largely abandoned a few decades later because of diminishing returns caused by repeated rediscovery of known compounds. More recently, it was proposed that the Waksman platform be revived, but with a focus on 'hidden' chemical diversity, for example by 'prospecting' in unusual environments, growing uncultured organisms, turning on silent operons containing biosynthetic genes, or metagenomic approaches (Lewis 2013). The merits of this suggestion were shown a short time later with the discovery of the antibiotic teixobactin, produced by a previously uncultured soil bacterium which was isolated using an iChip device (Ling *et al.* 2015). While not yet fully characterized, it is possible that some of the antimicrobial activities from these isolates, plus those identified in the metagenome (Tables S5 and S6), may be the result of novel antimicrobial compounds, and is the subject of ongoing study.

In addition to demonstrating the potential discovery of novel antimicrobials from this environment, we have previously published studies showing the identification and production of robust transaminases for biocatalysis in the pharmaceutical industry using the same collection of isolates, both bacterial and archaeal (Kelly *et al.* 2017, 2019), and the metagenomic sequence data (Kelly *et al.* 2018), demonstrating the potential for biodiscovery associated with this previously untapped microbiome. This salt mine environment facilitated the isolation of both bacteria and archaea from the same hypersaline source, making this a potentially important model system for studies involving both domains, for example those involving cross-domain signal induction (Zhang *et al.* 2012a; Megaw and Gilmore 2017) or horizontal gene transfer between members of the two domains inhabiting the same environment (Fuchsman *et al.* 2017). Based on the potential applications already described, the unique microbiome of Kilroot salt mine appears to represent an excellent resource with high potential for biodiscovery and for future fundamental and applied studies.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSLE* online.

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Conflicts of Interests. None declared.

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