Extreme environments: microbiology leading to specialised metabolites

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Abstract

The prevalence of multi-drug resistant microbial pathogens due to the continued misuse and overuse of antibiotics in agriculture and medicine is raising the prospect of a return to the pre-antibiotic days of medicine at the time of diminishing numbers of drug leads. The good news is that an increased understanding of the nature and extent of microbial diversity in natural habitats coupled with the application of new technologies in microbiology and chemistry is opening up new strategies in the search for new specialised products with therapeutic properties. This review explores the premise that harsh environmental conditions in extreme biomes, notably in deserts, permafrost soils and deep-sea sediments select for microorganisms, especially actinobacteria, cyanobacteria and fungi, with the potential to synthesise new druggable molecules. There is evidence over the past decade that microorganisms adapted to life in extreme habitats are a rich source of new specialised metabolites. Extreme habitats by their very nature tend to be fragile hence there is a need to conserve those known to be hot-spots of novel gifted microorganisms needed to drive drug discovery campaigns and innovative biotechnology. This review also provides

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Running headline: Microorganisms from extreme environments.
an overview of microbial-derived molecules and their biological activities focusing on the period from 2010 until 2018, over this time 186 novel structures were isolated from 129 representatives of microbial taxa recovered from extreme habitats.

Keywords: extremophiles, extreme environments, actinobacteria, cyanobacteria, fungi, specialised metabolites, biological activities.

Introduction

Microorganisms remain the most promising source of novel specialised (secondary) metabolites as the chemical diversity of these compounds cannot be matched currently by that of chemical libraries (Krug & Müller, 2014) hence the continued interest in the search for new natural products (NP) needed to drive innovative biotechnology (Katz & Baltz, 2016). In recent times, the quest for novel bioactive molecules, especially those that can be developed to control multidrug-resistant pathogens and treat chronic conditions such as cancer, dementia and epilepsy has been focused on the isolation, characterisation (taxonomy) and dereplication (assignment of isolates to taxonomically meaningful groups) of previously unknown microorganisms isolated from the extremobiosphere (Bull 2011; Horikoshi & Bull, 2011; Bull & Goodfellow, 2019).

The extremobiosphere encompasses a broad range of biomes that include hyper-arid deserts, deep-sea sediments and permafrost soils, as well as acid and high-temperature environments. Such extreme habitats are characterised by combinations of environmental variables such as anoxia, aridity, extreme temperatures, low concentrations of organic matter, high salinity and intense irradiation. The search for new bioactive compounds from the extremobiosphere rests on the premise that harsh abiotic conditions select for novel microorganisms that express new chemistry (Okoro et al., 2009). Microorganisms which live optimally under extreme conditions are considered to be extremophiles (Macelroy, 1974) whereas those that can tolerate such conditions are described as being extremotolerants.

It is well known that actinobacteria from the phylum Actinobacteria (sensu Goodfellow, 2012) have a unique capacity to synthesise new drug leads (Bérdy, 2012; Genilloud, 2017). These organisms, notably Streptomyces, account for around 70% of known antibiotics, including most of those in clinical use (Newman & Cragg, 2016). The recent discovery that filamentous actinobacteria with large genomes contain many natural product-biosynthetic gene clusters (NP-BGCs) which express for unknown drug leads partly accounts for the increased focus on these microorganisms in bioprospecting campaigns (Bull & Goodfellow, 2019). Especially gifted actinobacteria that have moderate or large genomes (~ 5.0-7.9 MB and > 8.0 MB, respectively) harbour many NP-BGCs (20-19 and > 30,
respectively) (Baltz, 2017, 2019, Nouiou et al., 2019) are at the premium in the search for new chemical leads using state-of-art-technologies, such as genome mining (Harvey et al., 2015; Goodfellow et al., 2018). Other microorganisms with large genomes laden with NP-BGCs include cyanobacteria (Vijayakumar & Menakha, 2015), ktedonobacteria (Zheng et al., 2019), myxobacteria (Hoffman et al., 2018) and fungi (Keller et al., 2005). Indeed, members of all of these taxa have been highlighted as potential candidates for drug discovery programmes (Dixit & Suseela, 2013, Micallef et al., 2015, Baltz, 2019, Keller, 2019). In light of these developments, it clearly makes good sense to focus bioprospecting campaigns on microorganisms with moderate to large genomes. Another important development is the realisation that in some actinobacterial taxa, such as the genera Amycolatopsis, Frankia and Micromonospora, there is a link between the distribution of NP-BGCs and phylogeny (Adamek et al., 2018, Carro et al., 2018a, Nouiou et al., 2019). In addition, information drawn from whole genome sequences is providing fascinating insights into how microorganisms adapt to extreme habitats, as witnessed by the ability of members of the actinobacterial family Geodermatophilaceae to withstand extreme environmental conditions that prevail in hyper-arid desert habitats (Castro et al., 2018a, 2018b).

The steps involved in culture-dependent natural product discovery pipelines are outlined in Figure 1. The initial step, the selection of environmental samples is followed by the selective isolation, dereplication and generation of microbial strain libraries. Subsequent steps involve the detection of bioactive compounds from representatives of strain libraries, primary screening of fermentation broths and associated biomass extracts, and chemical dereplication of extracts followed by secondary fermentation using production media. Structural determination of drug leads and biological testing of purified compounds is the final stage in this process. At all of these stages, the application of technological developments and associated bioinformatics procedures in microbiology, molecular biology and chemistry minimises the costly rediscovery of known compounds which until recently bedevilled bioprospecting campaigns (Baltz, 2017).

This review is designed to determine the extent to which microorganisms, notably actinobacteria, cyanobacteria and fungi isolated from diverse extreme habitats are a rich source of new drug leads of potential value in agriculture, industry and medicine. To this end, we not only discuss the microbiology of specific extreme habitats but also relationships between dereplicated microorganisms and their ability to synthesise diverse chemical classes of specialised metabolites.

Deserts

Until recently, deserts received little attention from microbiologists even though they account for around 20% of the landmass on the planet (Laity 2009). Desert habitats are challenging for microorganisms, notably the scarcity of
water. Several pilot studies have been carried out to establish the microbial flora of non-polar deserts (Kurapova et al., 2012; Tiwari et al., 2015; Ouchari et al., 2018) but the most extensive studies of cultivable microbial diversity in desert biomes have been focused on sites in the Atacama Desert in Northern Chile (Bull et al., 2016, 2018a, Bull & Goodfellow, 2019). The location and abiotic conditions associated with this temperate, non-polar desert have been the subject of several reviews (Azua–Bustos et al., 2012; Cordero et al., 2018) and its uniqueness highlighted (Bull et al., 2016). Microbial surveys of Atacama Desert soils and regoliths have been focused on the isolation and characterisation of microorganisms, notably actinobacteria, from hyper-arid and extreme hyper-arid regions where the mean annual rainfall to mean annual evaporation is 0.05 and 0.02%, respectively (Houston, 2006). These conditions are compounded by other factors such as the presence of inorganic oxidants, very low levels of organic carbon, extreme temperature fluctuations and intense solar radiation; the latter contributes, in a synergistic way, with desiccation to limit the growth and survival of microbial life (Gómez–Silva, 2018). Until recently, it was believed that such harsh abiotic conditions made any form of life in the core region of the Atacama Desert virtually impossible (Navarro – Gonzalez et al., 2003). However, it is now known that different groups of microorganisms have adapted to the extreme environmental conditions that prevail in the desert (Schultz–Makuch et al., 2018), so much so that a highly unusual rain event in the hyper-arid core of the desert led to the decimation of surface microbial communities (Azua-Bustos et al., 2018).

**Actinobacteria**

In recent times innumerable filamentous actinobacteria have been isolated from desert soils and assigned not only to the ubiquitous genus *Streptomyces* but also to rare and poorly studied taxa, such as the genera *Actinomadura, Amycolatopsis, Nocardiopsis, Nonomuraea, Saccharopolyspora* and *Saccharothrix* (Okoro et al., 2009, Goodfellow et al., 2018). The application of cutting edge taxonomic and screening methods, especially those based on whole genome sequence data; show that such filamentous actinobacteria are a significant source of novel specialised metabolites (Bull et al., 2016; Carro et al., 2018a, 2018b). In this context, especially gifted isolates from Atacama Desert habitats have been classified as novel species of *Streptomyces*, such as *Streptomyces asenjonii* (Goodfellow et al., 2017), *Streptomyces deserti* (Santhanam et al., 2012a), *Streptomyces atacamaensis* (Santhanam et al., 2012b), *Streptomyces bullii* (Santhanam et al. 2013), and *Streptomyces leeuwenhoekii* (Busarakam et al., 2014). Recently, presumptively gifted isolates from a high altitude Cerro Chajnantor gravel soil in northern Chile have been validly named as *Micromonospora acroterricola* (Carro et al., 2019), *Micromonospora anda*, and *Micromonospora inaquosa* (Carro et al., 2018a). Similarly, novel dereplicated isolates belonging to less well-known genera have been validly named, as exemplified by *Amycolatopsis vastitatis* (Idris et al. 2018), *Lentzea chajnantoensis* (Idris et al.
Pseudonocardia nigra (Trujillo et al. 2017). The genomes of some of these isolates have the capacity to synthesise many novel specialised metabolites and contain stress-related genes that provide an insight into how actinobacteria adapt to the extreme environmental conditions found in Atacama Desert habitats (Carro et al., 2018b; Idris et al., 2018).

A steady flow of filamentous actinobacteria recovered from other non-polar deserts have been validly named as new species, they include Amycolatopsis desertii (Busarakam et al. 2016a), Desertiactinospora gelatinilytica (Saygin et al., 2019), Nakamurella desertii (Liu et al., 2018), Prauserella isguensis (Saker et al., 2015), Saccharothrix algeriensis (Zitoune et al., 2004), Saccharothrix tharensis (Ibeyaima et al. 2018) and Streptosporangium becharense (Chaouch et al., 2016).

Actinobacteria that are amycelial or form rudimentary hyphae have received less attention though they are known to be common in desert soils (Idris et al. 2017b; Bull et al., 2018b). Members of the family Geodermatophilaceae have received the most attention, notably the genera Blastococcus, Geodermatophilus and Modestobacter; members of these taxa are known to be resistant to desiccation, ionising radiation, UV-light and heavy metals (Sghaijer et al. 2016). Dereplicated Geodermatophilaceae strains from Atacama Desert habitats have been validly named as Blastococcus atacamensis (Castro et al., 2018a), Geodermatophilus chilensis (Castro et al., 2018b) and Modestobacter caceresii (Busarakam et al., 2016b) and shown to have moderately large genomes (3.9 – 5.9 Mb). Representatives of these taxa not only contain NP-BGCs but also stress related genes that encode for properties such as carbon starvation, temperature fluxes, osmotic stress and UV-light. Other novel γ-radiation resistant geodermatophili have been isolated from the Sahara Desert, as exemplified by Geodermatophilus pulveris (Hezbri et al., 2016). In addition, radiation resistant actinobacteria belonging to the genera Agrococcus, Arthrobacter, Cellulomonas, Kocuria, Knoella and Nocardioides have been recovered from the Taklamakam Desert (Yu et al., 2015). Amycelial actinobacteria isolated from other deserts include Arthrobacter desertii (Hu et al., 2016), Citricoccus alkalitolerans (Li et al., 2005), and Kocuria aegyptia (Li et al., 2006). A particularly interesting development was the discovery that the sole member of the novel taxon Desertimonas flava which was recovered from the Gurbantünggüt Desert, a desert which occupies a large part of the Dzungarian Basin in northern Xinjiang Province, China, belongs to the newly proposed family Ilumatobacteraceae of the order Acidomicrobiales (Asem et al., 2018).

Alkali-halotolerant actinobacteria retrieved from salty hyper-arid soils have been shown to be adapted to alkaline, drought and extreme temperatures (Mohammadipanah & Wink, 2016). Streptomyces aburaviensis, an isolate from saline arid desert soil in Kutch, India grows well at pH 8.5 in the presence of 15% w/v NaCl (Thumar et al., 2010).
Mycetocola manganoxydans, a unique non-sporulating alkaliphilic actinobacterium isolated from Taklamakan Desert can oxidise manganese ions (Luo et al., 2012). In addition, several alkalihalophilic actinobacteria have been isolated from the Qinghai-Tibet Plateau, including members of the genera Cellulomonas, Saccharothrix and Streptosporangium (Ding et al., 2013) while Actinomadura and Nocardiopsis strains have been recovered from alkaline arid soils adjacent to salty lakes in Buryatiya (Labsanova et al., 2014). A novel alkaliphilic Streptomyces strain isolated from an alkaline soil in the arid area of Boho, N. Ireland was found to grow under intense γ-radiation (4 kGy) at pH 10.5, and shown to be a potential source of novel specialised metabolites that inhibit ESKAPE pathogens (Terra et al., 2018).

Cyanobacteria

These oxygenic photosynthetic prokaryotes, which form a single taxonomic and phylogenetic group, produce a broad range of bioactive compounds including ones that show anti-microbial, anti-protozoal and anti-inflammatory activity (Micallef et al., 2015, Vijayakumar & Menakha, 2015). Endolithic (rock-inhabiting) cyanobacteria dominated by Chroococcidiopsis species were first reported from the Negev Desert (Potts & Friedmann, 1981), but are now known to be common in desert biomes given their remarkable ability to cope with extreme aridity and solar radiation (Lacap–Bugler et al., 2017). Indeed, cyanobacteria dominate hypolithic (under rock) desert communities and may be major drivers of community assembly and function (Bahl et al., 2011). Microbial communities dominated by Chroococcidiopsis strains inhabit halite deposits in the hyper-arid core of the Atacama Desert (Wierzchos et al., 2006). It has also been shown that cyanobacteria and associated heterotrophic bacteria reside within pore spaces in nodule-shaped halite crusts (Ríos et al., 2010; Roldán et al., 2014). These lithic microbial communities have developed adaptive strategies to withstand the extreme environmental conditions that prevail in the Atacama Desert (Gomez-Silva, 2018), as is the case with similar communities in the Namib Desert (Stomeo et al., 2013). It is evident that green hypoliths in the hyper-arid core of the Atacama Desert are dominated by Chroococcidiopsis and Nostocales species and red hypoliths by Chloroflexi (Lacap et al., 2011) while Noctocophycideae and Synechococcophycideae species are present in lower numbers (Vitek et al., 2014). Cyanobacteria also produce pigments which may protect them against lethal doses of UV-radiation in hyper-arid habitats (Powell et al., 2015).

Fungi

Like actinobacteria and cyanobacteria, fungi show a range of adaptive mechanisms which allow them to withstand environmental extremes found in desert ecosystems (Onofri et al., 2007; Stevenson et al., 2017; Santiago et al., 2018), as shown by their ability to synthesise melanin pigments that provide protection against high levels of UV-
radiation (Gessler et al., 2014). Early isolation studies on soils from the Negev and Sonoran deserts revealed extensive fungal diversity (Taylor-George et al., 1983), results now known to square with the view that fungi are the most stress-resistant eukaryotes (Sterflinger et al., 2012), a point especially apt with respect to microbial rock fungi (Palmer et al., 1987; Gonçalves et al., 2016).

To date, fungi isolated from desert habitats have featured rarely in bioprospecting campaigns even though fungal taxa, including novel species, are common in desert landscapes. A preliminary survey of Atacama Desert soils by Conley et al. (2006), for instance, recorded 13 distinct fungal genera, notably Alternaria and Ulocladium species; it is now clear that fungal communities in this desert represent an unknown element of global fungal diversity (Santiago et al., 2018). Further, a survey of fungi from Makhtesh Ramon desert soil found 135 novel species dominated by ascomycetes (Grishkan & Nevo, 2010). In addition, 77 lichenoid fungal species detected along two altitudinal transects at Alto Patache in the Atacama Desert included four new species, Amandinea efflorescens, Diplodia canescens, Myriospora smaragdula and Rhizocarpon simillimum (Castillo & Beck, 2012), while high altitude rocks in the desert were a source of fungi belonging to the genera Cladosporium, Neucatenulostroma and Penicillium (Gonclaves et al., 2016). Further, two novel halophilic fungi isolated from a cave in the Coastal Range of the Atacama Desert were designated as Aspergillus atacamensis and Aspergillus salisburgensis (Martinelli et al., 2017). Extensive fungal diversity has been recorded in Middle East desert sand (Murgia et al., 2018) and three novel fungal species, Diverispora omaniana, Septoglomus nakheelum and Rhizophagus arabicus, were recorded from a desert in Oman (Symanczik et al., 2014).

Deserts-associated specialised metabolites

The range of microorganisms found in deserts are potential sources of new chemical diversity but have been understudied chemically, partly reflecting a view that desert microorganisms were likely to synthesise bioactive metabolites due to limited numbers of competing microbial species present in extreme biomes (Pettit, 2011). However, over the last twenty years, actinobacteria, cyanobacteria and fungi isolated from unusual or extreme desert habitats have been shown to have evolved unique metabolic pathways for the synthesis of novel bioactive specialised metabolites (Peng et al., 2011; Jančič et al., 2016). Indeed, by 2008, more than 100 specialised metabolites had been isolated and identified from microorganisms isolated from such habitats (Wilson & Brimble, 2009).

N-containing compounds

Since 2010, most investigated bioactive molecules have been reported from actinobacteria recovered from hyper-arid Atacama Desert soils, as shown in Figure 2. Streptomyces leeuwenhoekii strain C34 produces ansamycin-type
macroyclic polyketides, the chaxamycins A-D (1-4) together with the aminoglycoside antibiotics hygromycin A (5), 5”-dihydrohygromycin A (6), deferrioxamine E (nocardamine, 7) as well as desferrioxamine B (8), the latter were isolated from the same strain using the OSMAC (One Strain Many Compounds) approach (Rateb et al., 2011a; 2011b). Further examples of novel bioactive molecules from Atacama Desert streptomycetes include the aminobenzoquinones, abenquines A, B1, B2, C and D (9-13) which showed inhibitory activity against bacteria and dermatophytic fungi (Schulz et al., 2011). Fermentation of S. leeuwenhoekii strain C58 led to the isolation of chaxapeptin (14), a novel ribosomally-synthesised and post-translationally modified lasso peptide which exhibits significant in vitro inhibitory activity against human lung cancer cells (Elsayed et al., 2015). Recently, bio-guided fractionation of a culture broth extract of S. asenjonii strain 42.f (Goodfellow et al., 2017) led to the isolation of new bioactive polyketides of the rare β-diketone family, asenjonamides A–C (15-17) which showed potent antibacterial effects against Gram-positive bacteria., it is particularly interesting that asenjonamide C (17) shows comparable activity to that of tetracycline (Abdelkader et al., 2018). In the same study, a series of bioactive acylated 4-aminoheptosyl-β-N-glycosides, spicamycins A–E (18-22), were isolated in a pure form for the first time. These antibiotics were initially found as a non-separable mixture of seven compounds extracted from Streptomyces alanosinicus (Hayakawa et al., 1983). There is evidence that this unique class of nucleoside antibiotics may provide a promising lead for the development of new anticancer drugs (Gadgeel et al., 2003).

Cyanobacteria are widely distributed in both arid and hyper-arid ecosystems, and are the source of many bioactive compounds (Micaller et al., 2015; Singh et al., 2005). They produce pigments like scytonemin (23) and the mycosporine-like amino acids shinorine (24) and porphyra-334 (25) which may absorb potentially lethal doses of UV-radiation found in hyper-arid environments., these compounds are being evaluated as a source of potent sunscreens (Powell et al., 2015). Scytonemin (23), which is produced by a Chroococcidiopsis strain, has potential as a chemical marker for endolithic cyanobacterial colonies in halite crusts which are common in the hyper-arid zone of the Atacama Desert (Vítek et al., 2014). This compound is of considerable interest as it causes attenuation of mitogen-induced inflammatory hyperproliferation through inhibition of polo-like kinase hence its unique dimeric structure may prove to be a potential scaffold for the development of potent kinase inhibitors (D’Orazio et al., 2012). Recently, the mycosporine-like amino acids, shinorine (24) and porphyra-334 (25) were found to exhibit anti-inflammatory effects by interference with NF-κB activation (Becker et al., 2016).

Cyanobacteria found in extreme habitats, such as alkaline lakes, hyper-arid desert soils, hypersaline environments and polar regions, produce cyanotoxins, diverse specialised metabolites (e.g., alkaloids, amino acids and cyclic peptides) which can be classified into hepatotoxins, neurotoxins and cytotoxins (Metcalf & Codd, 2012).
Cyanotoxins detected in habitats such as these include the neurotoxins anatoxin-a (26), anatoxin-a(S) (27), β-methylamino-2-propionic acid (28), and 2,4-diaminobutyric acid (29), as well as the hepatotoxins, nodularin (30) and cylindrospermopsin (31). Biosynthesis of these toxins is mainly attributed to the cyanobacterial genera Arthrospira, Oscillatoria and Synechococcus (Cirés et al., 2017). Apart from their toxicological properties, cylindrospermopsin (31) and anatoxin-a (26) show estrogenic activity in vivo and in vitro assays (Liu et al., 2018).

Recent studies on fungi from desert ecosystems underlines their potential as prolific source of novel chemical entities which show interesting bioactivities (Santiago et al., 2018). Members of the genus Wallemia, a taxon which encompasses seven species, were previously considered to be halophilic (Zalar et al., 2005). However, Wallemia sebi, the sole representative of the genus isolated from the Atacama Desert produces a range of specialised metabolites such as wallimidione (32) (Desroches et al., 2014), 15-azasterol (33) and 24,28-dihydro-15-azasterol (34) (Jančič et al., 2016). Molecules 33 and 34, also known as UCA1064-A and UCA1064-B, respectively exhibit in vivo antitumor activity against a mouse mammary tumour model and in vitro antiproliferative activity against HeLa S3 cells; they also show antifungal activity against Saccharomyces cerevisiae and inhibit Gram-positive bacteria (Jančič et al., 2016). Furthermore, the alkaloid cyclopentanopyridine (35) isolated from a halophilic strain of W. sebi inhibits Enterobacter aerogenes (Peng et al., 2011).

Macrolides

Streptomyces leeuwenhoekii C34† from Atacama Desert soil produces chaxalactins A-C (36-38), a rare class of 22-membered macrolactone polyketides which show antibacterial activity against Gram-positive bacteria (Rateb et al., 2011a; 2011b). In turn, S. leeuwenhoekii strain C38, isolated from the same desert location, synthesises 22-membered macrolactone antibiotics, the atacamycins A-C (39-41) (Nachtigall et al., 2011).

Other metabolites

Chemical investigation of Lentzea chajnantorensis strain H45 recovered from high-altitude Atacama Desert soil (Idris et al., 2017a) led to the characterisation of new diene glycosides, lentzeosides A-F (42-47) which show inhibitory activity against HIV integrase (Wichner et al., 2017). Further, the Atacama Desert-derived fungus, W. sebi produces two unique terpenes, walleminone (48) and walleminol (49) (Jančič et al., 2016).

Permafrost soils

Permafrost is the layer below the earth’s crust that has remained at or below 0°C for at least two consecutive years (Jansson & Taş, 2014). It is estimated that approximately 25 % of the landmass of the planet is underlayen by permafrost soils. These soils, together with overlying (naturally thawing) layers, provide unique habitats for active, cold-adapted, microbial communities (Bakermans et al., 2014; De Maayer et al., 2014; Hu et al., 2015).
for cells that have remained viable for millions of years (Gilichinsky et al., 2008). Indeed, according to the Snow
Ball Earth hypothesis, the planet has been completely or almost completely frozen at least once within the last 650
million years suggesting that microorganisms would have had to adapt to sub-zero temperatures to survive (Schop &
Klein 1992). Permafrost can be distinguished from other low temperature biomes, such as sea ice and deep oceans,
by its structural heterogeneity given horizontal and vertical differences in soil, ice and organic matter content
(Jansson & Taş, 2014).

Microorganisms adapted to temperatures that range from -17°C to +10°C are referred to as cryophiles (Feller &
Gerday 2003). Water availability and temperature are the most important abiotic factors influencing microbial
diversity in cold environments though high viscosity and low thermal energy offer additional challenges (Jansson &
Taş, 2014). Nucleic acid replication, transcription and translation are inhibited under such conditions while proteins
can denature causing loss of cell membrane fluidity (Chattopadhyay, 2006; D'Amico et al., 2006). Microorganisms
adapted to life in such cold environments have developed several strategies for survival, such as initiating dormant
states and generating specialised metabolites and proteins (Bakermans et al., 2009). Cryophilic microorganisms can
also reduce their metabolism and nutrient uptake by storing energy as polyphosphates, triglycerides, wax esters and
glycogen (Bowman, 2008) and can adapt their cellular structure by regulating branched and saturated fatty-acid
production to maintain cell membrane fluidity at freezing temperatures (Unell et al., 2007).

**Prokaryotes**

Arctic permafrost soils tend to be dominated by Acidobacteria, Actinobacteria, Cyanobacteria, as well as by
Proteobacteria belonging to the Burkholderiales (β-proteobacteria), Myxococcales (δ-proteobacteria), Rhizobiales
(α-proteobacteria) and Xanthomonadales (γ-proteobacteria) (Malard and Pearce, 2018). These authors suggested
that biogeographic variation may be a feature of Arctic soils as actinobacteria occurred in lower numbers in
Greenland and Finland while much higher populations of Bacteroidetes were evident in Alaskan, Canadian and
Svalbard permafrost. These abundant groups also included Chloroflexi, Cyanobacteria, Firmicutes,
Gemmatimonadetes, Planctomycetes and Verrucomicrobia. In turn, the dominant taxa found in Chinese permafrost
soils were Actinobacteria, Firmicutes and Proteobacteria (Hu et al., 2015). The dominant cyanobacterial orders in
Arctic soil crusts were found to be the Nostocales, Oscillatoriales and Synechococcales (Steven et al., 2013;
Pushkareva et al., 2015). Indeed, cyanobacteria are mainly responsible for the uptake of CO_2 and N_2 as plants in
Arctic ecosystems are unable to fix nitrogen (Malard & Pearce, 2018).

Regional populations of cyanobacteria may also be a feature of Antarctic soils (Namsaraev et al., 2010). Archaea
have also been isolated from permafrost (Jansson & Taş, 2014). Archaeal communities in Arctic soils seem to be
variable through *Methanobacteria* and *Methanomicrobia* (*Euryarchaeota*) are abundant in Alaskan and Greenland soils (Malard and Pearce, 2018) while isolates related to the genera *Methanolobus* and *Methanomethylovorans* have been recovered from frozen ground in the Zoige wetland of the Qinghai – Tibet plateau (Zhang et al. 2008). The methane released by these organisms can be used as a sole carbon source by methanotrophic bacteria, such as α- and γ-proteobacteria (Coolen et al., 2011) and members of the *Methanococcales* (Martineau et al., 2014).

**Fungi**

In general, fungal communities in Arctic permafrost are dominated by *Ascomycota*, *Basidiomycota* and *Chytridiomycota* (Gittel et al., 2014; Zhang et al., 2016), through the survival of associated shrubs are dependent on ectomycorrhizal fungi (Deslippe et al., 2011; Fujiyoshi et al., 2011). In turn, common genera include *Aspergillus*, *Cladosporium*, *Geomyces* and *Penicillium* (Ozerskaya et al., 2009). Over 400 taxonomically distinct genera have been recovered from Antarctic soil systems suggesting that fungi may be the most diverse biota in this milieu (Bridge & Spooner 2012); isolates from Cape Royds, Antarctica keyed out to the genera *Cadospora*, *Geomyces* and *Thielava* (Blanchette et al., 2010). Indeed, the ascomycete genera *Cadospora* and *Geomyces* may be endemic to Antarctic soils (Arenz & Blanchette 2011). Fungal diversity in Antarctic lichens from King George Island was shown to include the *Arthonimycetes*, *Eurotiomycetes*, *Leoanoromycetes*, *Leotiomycetes* and *Sordariomycetes* (*Ascomycota*) and the *Cystobasidiomycetes* and *Tremellomycetes* of the *Basidiomycota* (Park et al., 2015).

**Permafrost soils-associated specialised metabolites**

Their rapid growth rate and ability to metabolise a wide array of substrates have enabled fungi to become the dominant microorganisms in polar habitats. Two new epipolythiodioxopiperazines, the chetracins B and C (50, 51), together with five new diketopiperazines, oidioperazines A–D (52–55) and chetracin D (56) were isolated from the Antarctic psychrophilic fungus *Oidiodendron truncatum*. Chetracin B (50) shows potent anticancer activity at a nanomolar concentration against a panel of human cancer cell lines while chetracin C (51) displayed a significant effect at a micromolar concentration suggesting that the sulfide bridge is an essential structural feature for the activity of these compounds (Li et al., 2012). Another psychrophilic fungus, *Eutypella* sp. D1, isolated from an Arctic soil on London Island of Fongsfjorden, yielded the new cytochalasins Z_{24}, Z_{25}, Z_{26} (57–59). Cytochalasin Z_{24} (57) showed moderate cytotoxicity toward human breast cancer MCF-7 cells (Liu et al., 2014). Further chemical profiling of the *Eutypella* strain led to the isolation of two new N-containing diterpenes; libertellenone G (60) and eutypenoid B (61). The former exhibited significant antibacterial activity against Gram-positive and Gram-negative
bacteria and the latter potent immunosuppressive activity (Lu et al., 2014; Zhang et al., 2016). Lindgomycin (62), an unusual polyketide with a novel carbon skeleton, and ascosetin (63) were recovered from an Arctic sponge-derived fungal strain and classified in the family Lindgomycetaceae. All of these metabolites exhibited potent antimicrobial activities against several pathogenic Gram-positive bacteria, including MRSA and the pathogenic yeast Candida albicans (Ondeyka et al., 2014; Wu et al., 2015).

The new benzoxazine glycosides, arcticoside (64) and C-1027 chromophore-V (65) derived from an Arctic marine Streptomyces strain showed inhibitory activity against Candida albicans isocitrate lyase and breast and colorectal carcinoma cells. Additionally, C-1027 chromophore-III (66) and fijiolides A and B (67, 68) were isolated from the same strain (Moon et al., 2014). An Arctic freshwater-derived bacterium, Pseudomonas fluorescens BD5 produced unusual cyclic lipopeptide biosurfactants, namely pseudofactin I and II (69, 70), the structure of which is unique as a palmitic acid is connected to a terminal peptide moiety of eighth amino acids. Interestingly, the stability and emulsification activity of these metabolites were greater than those of the conventional synthetic surfactants Triton X-100 and Tween 20 suggesting that they may be of potential value in bioremediation and biomedicine (Janek et al., 2010).

**Polyketides**

Two highly oxygenated polyketides, penilactones A and B (71, 72), isolated from an Antarctic deepsea-derived fungus, Penicillium crustosum, inhibited the nuclear factor-κB (NF-κB) (Wu et al., 2012). Further, a psychrophilic fungal strain isolated from an Antarctic marine sponge and assigned to the genus Pseudogymnoascus produced four new nitroasterric acid derivatives, pseudogymnoascins A–C (73–75) and 3-nitroasterric acid (76); these polyketides are the first nitro-derivatives of the known fungal metabolite asterric acid. In general, most reported asterric acid derivatives have been found to show antibacterial and antifungal activity but the present nitro-derivatives were inactive possibly due to the presence of the nitro group (Figueroa et al., 2015). Bio-guided fractionation of the culture broth of the Antarctic soil-derived Aspergillus ochraceopetaliformis strain led to the isolation of five new highly oxygenated polyketides, ochraceopones A–E (77–81), along with a new double bond isomer of asteltoxin (82), isoasteltoxin (83), as well as asteltoxin B (84). Among these metabolites, ochraceopones A (77) and isoasteltoxin (83) showed promising antiviral activity against the influenza viruses H1N1 and H3N2 (Wang et al., 2016).

**Terpenes**

A psychrophilic fungal Eutypella strain isolated from an Arctic soil on London Island was the source of four new diterpenes: scoparasin B (85), libertellenone H (86) and eutypenoids A and C (87, 88) (Liu et al., 2014). In turn, the
The Antarctic moss-derived fungus *Penicillium funiculosum* showed week inhibitory activity against influenza A virus H1N1 (Zhou et al., 2015).

**Lipids**

Screening for new anti-*Burkholderia cepacia* complex compounds from bacteria isolated from the Ross Sea (Antarctica) led to the isolation of three new rhamnolipids (91-93) from *Pseudomonas* strain BNT1 (Tedesco et al., 2016).

**Deep-sea sediments**

Taxonomically diverse microorganisms able to synthesise bioactive metabolites are common in marine habitats (Goodfellow & Fiedler, 2010; Wang et al., 2016). In contrast, partly for logistics reasons, less attention has been given to the microflora of deep-sea sediments, notably those in polar regions and oceanic trenches. However, it is now becoming apparent that bacteria and fungi adapted to extreme conditions in these habitats, notably low temperatures (-1 to 4°C), dearth of nutrients and high hydrostatic pressure, provide interesting targets for bioprospecting campaigns (Zhang et al., 2014; Goncalves et al., 2017; Dickinson et al., 2016).

**Bacteria**

Arctic marine sediments contain diverse actinobacterial communities including representatives of putatively novel species (Zhang et al., 2014) whereas samples taken along a transition between the Atlantic Peninsula and South America were dominated in terms of genetic diversity by γ-proteobacteria and euryarchaeota (Lopez-Garcia et al., 2001). Further, subsea floor core samples from the Nakau and Okinawa Troughs showed a range of halotolerant actinobacteria related to well-known antibiotic producing genera, such as *Nocardia*, *Pseudonocardia* and *Streptomyces* (Ulanova & Goo, 2015). These results are in good agreement with those of earlier studies on actinobacteria isolated from deep-sea sediments, including ones recovered from the Challenge Deep of the Mariana Trench (Pathom-aree et al., 2006). Neither cyanobacteria nor archaea are common in polar marine deep sediments (Dickinson et al., 2016).

**Fungi**

Taxonomically diverse marine fungi are a feature of cold deep-sea sediments. Seawater sediments taken from across the northern Antarctic peninsula, for instance, contained diverse fungal assemblages despite the harsh environmental conditions (Goncalves et al., 2017), as is the case with deep sea sediments from the East Indian Ocean (Zhang et al., 2014). Dominant cold-adapted yeasts from the deep Polar Sea include *Candida*, *Cryptococcus*, *Pichia* and *Rodotorula* spp. (Nagano et al., 2013). In addition, filamentous yeasts and fungi belonging to the
Ascomycota have been isolated from deep sea sediments from the Central Indian Basin (Singh et al., 2010, 2012).

Many Graphium spp. that displayed barotolerance at 100-bar pressure were recovered from Northern Antarctic Peninsula (Gonçalves et al., 2017).

**Deep-sea sediments-associated specialised metabolites**

**N-containing compounds**

Regulatory genes such as whiB-like (wblA50) play significant roles in actinobacterial specialised metabolism. Inactivation of wblA50 and vioB from a cold deep-sea-derived Streptomyces somaliensis strain led to spectacular changes in the production of specialised metabolites, notably in the synthesis of the new antimycin-type depsipeptide, somalimycin (94), and two analogues, USF-19A and urauchimycin D (95, 96) (Li et al., 2017).

Chemical investigation of Marinactinospora thermostolerans, Streptomyces scopuliridis and Streptomyces drozdowiczii strains recovered from deep-sea samples collected off South China (3865 m) led to the isolation of the cyclic peptides, marthiapetide A (97), desotamide (98), desotamide B-D (99-101) and marfomycins A-F (102-107) all of which showed antimicrobial activity against pathogenic Gram-positive bacteria (Zhou et al., 2012, 2014; Song et al., 2014). Additionally, three spirotetronate polyketides, lobophorin E (108), F (109) and H (110) extracted from two Streptomyces strains isolated from the same location at a depth of 2134 m, were shown to have antibacterial activity against a panel of Gram-positive bacteria (Niu et al., 2011; Pan et al., 2013). Further, a novel piezotolerant actinobacterium, Dermacoccus abyssi, isolated from Mariana Trench sediment produced novel phenazine metabolites, dermacozines A-J (111-120); dermacozines F (116) and G (117) showed interesting antiproliferative activity towards the leukaemia cell line K562 with IC50 values of 9 and 7 mM (Abdel-Mageed et al., 2010; Wagner et al., 2014). Chemical investigation of the Antarctic deep-sea fungus Penicillium sp. PR19N-1 led to the isolation of a new rare lactam-type metabolite, eremophilane (121) (Lin et al., 2014). Fermentation of another deep-sea fungus, Aspergillus westerdijkiae DFFSC013, led to the isolation of two new benzodiazepine alkaloids circumdatins K and L (122, 123), and two new indole alkaloids, 10-epi-sclerotiamide and 5-chlorosclerotiamide (124, 125) together with the novel amide, aspergilliamide B (126) (Peng et al., 2013).

**Terpenes**

A novel chloro-trinoreremophilane sesquiterpene (127) and three new chlorinated eremophilane sesquiterpenes (128-130) were also obtained from the Antarctic deep-sea fungus Penicillium sp. PR19N-1, these compounds showed moderate cytotoxic activity against the cancer cell lines HL-60 and A549 (Wu et al., 2013). Further chemical investigation on this strain led to the isolation of five new cytotoxic eremophilane-type sesquiterpenes (131-135) (Lin et al., 2014).
Polyketides

Five new α-pyrone derivatives, violapyrone A-C, J and H (136-140), which showed anti-MRSA activity were isolated from *Streptomyces somaliensis* strain isolated from a deep-sea sediment (Huang et al., 2016). Extensive chemical profiling of the Antarctic deep-sea fungus *Penicillium crustosum* PRB-2 resulted in the identification of penilactones A and B (141, 142), two novel polyketides with unusual highly oxygenated structures, along with known phenolic metabolites (Wu et al., 2012).

Highly acidic habitats

In general, extreme acidophiles can be defined as microorganisms that grow optimally at pH values below 3 (Johnson & Quatrini, 2016). These microorganisms are common in acid lakes, acid sulfate soils and acid mine wastes (Druschel et al., 2004; Mirete et al., 2017). Most studies have been focused on the structure and function of microbial communities in acid mine drainage (AMD) systems given their simplicity from a biological and geochemical perspective (Denef et al., 2010). Primary environmental variables that shape AMD habitats are dissolved metal concentrations, total organic carbon, dissolved oxygen, pH and temperature (Méndez-García et al., 2015). These factors, notably pH, help to drive microbial diversity patterns in acid mine wastes (Kuang et al. 2013).

Acidophilic algae, archaea, bacteria and fungi have developed ways of thriving or tolerating conditions in acidic biomes (Baker-Austin and Dopson 2007; Denef et al., 2010), as exemplified by roles played by cell membranes in archaea and bacteria (Konings et al., 2002; Falteisek & Čepička 2012).

Prokaryotes

Individual acidic habitats tend to be dominated by one or a few species (Mueller et al., 2010), as illustrated by the dominance of *Acidithiobacillus thiooxidans* on the ceilings of acidic caves (Ziegler et al., 2013); this extreme acidic chemolithotroph, which is a member of the order *Acidothiobacilliales* (Williams & Kelly 2013), is a feature of acid mine wastes across the world (Hedrich & Johnson 2013). Further, acidic, warm ferruginous mine wastes are dominated by *Ferroplasma* and *Leptospirillum* spp. (Denef et al., 2010) and their cold counterparts by *Acidithiobacillus thiooxidans* (Liljeqvist et al., 2015). In turn, *Leptospirillum* species are common members of acidophilic communities that catalyse the oxidation of ferrous ion (Goltsman et al., 2013). However, the primary bacterial lineages found in acid mine wastes are the *Acidobacteria*, *Actinobacteria*, *Aquificae*, *Firmacutes*, *Nitrospora*, *Proteobacteria* and *Candidatus* division TH7 (Chen et al., 2016). Predominant sulfate reducing bacteria detected in AMD systems include *Desulfurella*, *Desulfomonile*, *Syntrophobacter* and *Thermodesulfolobium* spp. (Sánchez-Andrea et al., 2012). Taxonomically diverse archaea present in acid mine wastes include *Acidianus*, *Metallophaera*, *Sulfolobus* and *Sulfurisporae* spp. (phylum *Crenarchaeota*) and the genus *Ferroplasma* of the
phylum *Euryarchaeota* (Chen *et al.*, 2015, 2016). Extremely acidophilic archaia are classified within the phylum *Euryarchaeota* (such as a *Picrophilus* sp. which is considered the most acidophilic of all known life-forms) and *Crenarchaeota* phyla have been regarded as thermoacidophiles (Aguilera *et al.*, 2016).

**Eukaryotes**

Relatively little is known about algae and fungi in acidic habitats even though they are an integral part of microbial communities (Falteisek & Čepička 2012). However, *Penicillium* spp. isolated from an abandoned open-pit containing acid-metal waste in Montana, USA were found to produce interesting, novel bioactive compounds (Zhang *et al.*, 2018) while algae from acid mine waste have been identified as *Chlorella protothecoids var. acidicola* and *Euglena mutabolis* (Johnson, 2012).

**Acidic habitats-associated specialised metabolites**

### Polyketides

In 2012, azaphilone-type polyketides, berkazaphilones A and B (143, 144) together with berkazaphilones C (145), berkedienoic acid (146), berkediolactone (147), vermistatin (148), dihydrovermistatin (149), penisimplicissin (150), and methylparaconic acid (151) were isolated from an extremophilic fungus recovered from the acid mine waste lake in Montana and identified as *Penicillium rubrum*. Berkazaphilones B and C (144, 145) and penisimplicissin (151) exhibited selective inhibitory activity against leukemia cancer cell lines through inhibition of caspase-1 (Stierle *et al.*, 2012a). An extremophilic fungus assigned to the genus *Pleurostomophora* isolated from the same acid mine lake produced three new azaphilones, berkchaetoazaphilones A-C (152-154) and the red pigment berkchaetorubramine (155..), berkchaetoazaphilone B (153) showed in vitro anti-inflammatory activity by inhibiting the production of IL-1β, TNFα, and IL-6 inflammatory mediators, and exhibited potent cytotoxic effects against human retinoblastoma, leukaemia and melanoma cell lines (Stierle *et al.*, 2015).

### Terpenes

The *Penicillium rubrum* strain mentioned above also produced interesting meroterpenoids, namely berkeleyones A-C (156-158), berkeleydione (159) and berkeleytrione (160), as well as preaustinoid A and A₁ (161, 162),. these metabolites inhibited the in vitro production of interleukin 1-β (Stierle *et al.*, 2011). Moreover, two new drimane sesquiterpene lactones, berkderimanes A and B (163, 164) and a new tricarboxylic acid (165) were isolated from another extremophilic fungal strain isolated from the Montana acid mine lake and identified as *Penicillium solitum*, berkderimanes A and B (163, 164) showed in vitro anti-inflammatory activity as they inhibited the enzymes caspase-1 and 3 (Stierle *et al.*, 2012b).

**Saline and hypersaline habitats**
Saline habitats can be considered as ones where salt concentrations correspond to the level found in seawater (3.5% w/v of total dissolved salts., Díaz-Cárdenas et al., 2017) whereas high salt environments have concentrations of salts >100 g/L. (Enache et al., 2017) as found in Antarctic biomes and hypersaline lakes. Halophilic microorganisms thrive under harsh environmental conditions that prevail in such habitats as they have evolved molecular and cellular mechanisms to cope with factors such as osmotic pressure and low water activity (Oren 1999; Gunde-Cimerman & Zalar 2014; Waditee-Sirisattha et al., 2016) whereas their halotolerant counterparts generally grow in the absence of salt but can tolerate high salt concentrations. Halophilic microorganisms, in particular, are being seen as a source of novel bioactive compounds (Waditee-Sirisattha et al., 2016; Díaz-Cárdenas et al., 2017).

**Prokaryotes**

Phylogenetically diverse halophilic and highly halotolerant archaea are common in hypersaline systems and include members of the class *Halobacteria* (Andrei et al., 2012). Extreme halophilic archaea have been isolated from hypersaline lakes in the Transylvanian Basin close to the salt minos of Turda (Baricz et al., 2014, 2015). Novel archaea isolated from commercial salt include *Halarchaeum acidophilum* (Minegishi et al., 2010) and *Natronoarchaeum mannanlyticum* (Shimane et al., 2010). Common bacteria recovered from hypersaline habitats have been assigned to the phyla *Actinobacteria* (*Streptomyces*), *Bacteriodetes* (*Flavobacteria*), *Cyanobacteria*, *Firmacutes* (*Bacilli* and *Clostridia*) and *Proteobacteria* (α and γ-proteobacteria) (Enache et al., 2017). Archaea and bacteria are also common in ancient halite (Jaakkola et al., 2016).

**Eukaryotes**

Extremely halotolerant and halophytic fungi have been isolated from biomes in solar salterns across the world, as exemplified by melanized members of the genera *Aspergillus*, *Cladosporium*, and *Penicillium* spp., and *Emericella* and *Eurotium* spp., non-melanised yeasts and *Wallemia* spp. (Gunde-Cimerman and Zalar 2014). Unclassified eukaryote have been detected in a hypersaline sulfate lake (Pontefract et al., 2017).

**Saline and hypersaline habitats-associated specialised metabolites**

**N-containing compounds**

Chemical investigation of *Streptomyces* strains derived from a saltern in Shinui Island (Republic of Korea) led to the isolation of a new chlorinated manumycin, salternamide A (166) and a new indolosesquiterpene, xiamycin D (167). Salternamide A (166) showed potent cytotoxicity against human colon and gastric cancer cell lines while xiamycin D (167) exhibited potent antiviral activity against the porcine epidemic diarrhoea virus (Kim et al., 2015). Chemical profiling of bioactive specialised metabolites from a *Bacillus* strain isolated from a saltern in Incheon, Korea led to the isolation of three new lipopeptides, iturin F₁ (168), iturin F₂ (169) and iturin A₉ (170), together with iturin A₈...
All of these compounds showed potent activity against pathogenic fungi and moderate antiproliferative activity against HeLa and srcs-NRK cell lines. Moreover, an in-vitro enzymatic assay of iturin A demonstrated significant inhibitory activity towards indoleamine 2,3-dioxygenase (Son et al., 2016).

Carotenoids

A Halobacterium salinarium strain was found to produce a group of potent antioxidant carotenoids, namely bacterioruberin (172), bisanhydrobacterioruberin (173), and trisanhydrobacterioruberin (174) (Mandelli et al., 2012).

High-temperature environments

Thermophilic and hyperthermophilic microorganisms are common in hot-springs and deep-sea hydrothermal vents, but are also found in artificial habitats such as compost (Rastogi et al., 2010; Urbieta et al., 2015). The optimal growth temperature for thermophiles is around 55°C and that for hyperthermophilic is above 80 °C though other extreme variables, such as low pH and high salt concentrations may affect their distributions. Hyperthermophilic microorganisms contain polyamines, these long chain functional polymers contribute to their survival at high temperatures (Hidese et al., 2018). Heat loving microorganisms continue to attract the interest of biotechnologists, notably as a source of liquid bio-fuels and thermostable enzymes (de Miguel Bouzas et al., 2006; Rastogi et al., 2010; Urbieta et al., 2015; Goh et al., 2013; Zeldes et al., 2015). Urbieta and her colleagues have spelt out the advantages of using thermophilic microorganisms in biotechnological processes.

Prokaryotes

Archaea and bacteria are common in high temperature environments, notably hot-springs. Common thermophilic bacteria include members of the genera Anoxybacillus, Geobacillus, Miobacillus and Thermus while their hyperthermophilic counterparts belong to the genera Aquificae and Thermatoga of the families Aquificaceae and Thermatogaceae, respectively (Urbietal., 2015). The majority of hyperthermophiles are archaea, as exemplified by the genera Desulfurococcus, Pyrodictium, Pyrococcus, Pyrolobus, Sulfolobus, Thermophylum and Thermoproteus of the Crenarchaeota (de Miguel Bouzas et al., 2006; Zeldes et al., 2015). Actinobacteria have been detected in hot springs, including representatives of the genera Couchiplanes, Glycomyces and Mycobacterium (Valverrde et al., 2012) and Actinospica, Amycolatopsis and Rhodococcus strains (Kusuma & Goodfellow, pres.com.).

Fungi

Thermophilic fungi received little attention though strains keyd out as Aspergillus clavatus (Jiang et al., 2013) and Talaromyces thermophilus (Chu et al., 2010; Guo et al., 2011) have been reported.

High-temperature environments-associated specialised metabolites

N-containing compounds
Clavatustides A and B (175, 176), two unusual cyclodepsipeptides containing an anthranilic acid dimer and phenyllactic acid residues were isolated from an *Aspergillus clavatus* strain associated with the thermophilic crab, *Xenograpsus testudinatus* which lives around the sulphur-rich hydrothermal vents in Taiwan, these compounds inhibit the proliferation of hepatocellular carcinoma cell lines (HepG2) by arresting their growth at G1 phase (Jiang *et al.*, 2013). Chemical investigation of the thermophilic fungus *Talaromyces thermophilus* afforded two new prenylated alkaloids talathermophilins A and B (177, 178) which showed nematicidal toxicity against the parasitic worm *Panagrellus redivivus* (Chu *et al.*, 2010). Subsequently, four talathermophilins (179-182) were isolated from the same fungus (Guo *et al.*, 2011).

**Carotenoids**

The thermophilic bacillus, *Thermus filiformis* was found to produce a group of potent antioxidant carotenoids, the all-trans-zeaxanthin (183), zeaxanthin monoglucoside (184), thermozeaxanthins (185) and thermobiszeaxanthins (186) (Mandelli *et al.*, 2012).

**Conclusions**

The microbiological and chemical data drawn from the recent literature on bioprospecting in selected extreme biomes provides strong backing for the premise that the harsh environmental conditions which prevail in the extreme biomes select for microorganisms that express new chemistry thereby opening up opportunities for therapeutic drug process development. Indeed, by the end of 2009, approximately 221 secondary metabolites were identified from diverse extremophilic microorganisms inhabiting different extreme habitats. However, over a subsequent eight-year period, taxonomically diverse extremophilic and extremotolerant microorganisms were the source of nearly 200 new specialised metabolites (Figure 6), the most frequently isolated classes of secondary metabolites were N-containing compounds and polyketides, many of which were produced by filamentous actinobacteria and fungi (Figure 7). It is also encouraging that these microorganisms synthesised a broad range of bioactive compounds with a skew towards the production of antibacterial and anticancer agents (Figure 8). In turn, microorganisms isolated from permafrost soils synthesised a broader range of chemical compounds than those from the other extreme habitats (Figures 2-5). In contrast, strains isolated from all of the extreme biomes, apart from the highly acidic habitats, were the source of many N-containing metabolites such as alkaloids and peptides. However, relatively little activity was shown by microorganisms isolated from saline and hypersaline habitats or from high temperature environments. In this context, it would be interesting to establish the extent of microbial diversity within and between diverse extreme biomes using culture-dependant procedures to determine the distribution of gifted microbial taxa *sensu* Baltz (2017, 2019).
The results of this survey provide further evidence of the value of the taxonomic approach to the discovery of new
drugs, as outlined in Figure 1. It is evident from Table 1 and 2 that novel species of filamentous actinobacteria and
fungi, particularly those isolated from Atacama Desert habitats, are a very good source of new bioactive compounds
thereby underpinning the view that these microorganisms should feature strongly in bioprospecting campaigns (Bull
& Goodfellow, 2019). Substantial improvements in the taxonomic approach to drug discovery can be expected given
developments in the classification and identification of eukaryotes and prokaryotes driven by advances in whole
genome sequencing procedures and associated improvements in bioinformatics as exemplified by Nouiou et al.
(2018), increased understanding of the extent of microbial diversity in natural habitats (Bull et al., 2018b) coupled
with developments designed to provide chemical dereplication (Bull & Goodfellow, 2019).
It can also be anticipated that improvements in search and discovering pipelines will be promoted in no small
measure by focusing heavily on novel culturable microorganisms with large genomes as advocated by Baltz (2017,
2019). In this context, future bioprospecting campaigns should not only be focused on microorganisms like
actinobacteria and fungi that are known to have a prosperity to synthesise antibiotics of therapeutic value (Bérdy,
2012, Newman & Cragg, 2016, Zhang et al., 2018), but also on representatives of under-explored microorganisms
with genomes rich in NP-BGCs, such as cyanobacteria, frankiae, ktedonobacteria and myxobacteria, representatives
of all of these taxa are known to be attractive candidates for drug discovery programmes (Dixit & Suseela, 2013,
Micallef et al., 2015, Baltz, 2019, Nouiou et al., 2019).
It can be concluded that there are strong grounds for believing that microbial natural products will continue to be a
source of new therapeutic agents, on optimism we share with others (Genilloud, 2017, Baltz, 2017, 2019, Bull &
Goodfellow, 2019). In particular, there are good reasons for believing that extremophilic and extremotolerant
microorganisms will have pride of place in the provision of a new generation of clinically significant drugs thereby
preventing a return to pre-antibiotic days of medicine. However, the success of future bioprospecting campaigns will
depend upon access to extreme biomes, notably ones like the Atacama Desert, that are known to be reservoirs of
gifted microorganisms, especially actinobacteria and cyanobacteria (Bull et al., 2016, Goodfellow et al., 2018, Bull
& Goodfellow, 2019). However, extreme habitats by their very nature are fragile and hence vulnerable to human
activities such as mining and to climate breakdown, as shown by the decimation of microbial communities in hyper-
arid core areas in the Atacama Desert due to unprecedented rain (Azua-Bustos et al., 2018) and the melting of
permafrost soils (Mackelprang et al., 2011, Hultman et al., 2015). Although concerns along these lines are revisited
from time to time (Cockell & Jones, 2009, Bull & Goodfellow, 2019). There is a crying requirements for
microbiologists and associated institutions to ensure that policy makers promote microbial conservation, especially in habitats known to be the source of gifted microorganisms.

Conflict of Interest

The authors have no conflict of interest.

References


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**Figure captions:**

**Figure 1.** Culture-dependent bioprospecting strategy (modified from Goodfellow & Fiedler, 2010).

**Figure 2.** Specialised metabolites derived from actinobacteria (red colour), cyanobacteria (blue colour) and fungi (black colour) recovered from desert habitats (compounds 1-49).

**Figure 3.** Specialised metabolites derived from actinobacteria (red color), bacteria (green colour) and fungi (black colour) isolated from permafrost soils (compounds 50-93).

**Figure 4.** Specialised metabolites derived from actinobacteria (red colour) and fungi (black colour) isolated from deep-sea sediments (compounds 94-142).

**Figure 5.** Specialised metabolites derived from actinobacteria (red color), bacteria (green colour) and fungi (black colour), isolated from acidic, hypersaline, and high temperature habitats (compounds 143-186).

**Figure 6.** Distribution of specialised metabolites synthesised by microorganisms isolated from extreme habitats before and after 2010.

**Figure 7.** Classes of specialised metabolites produced by microorganisms isolated from extremophile habitats.

**Figure 8.** Bioactivities of specialised metabolites produced by microorganisms recovered from diverse extremophile habitats.
Table 1. Examples of actinobacteria isolated from selected extreme biomes and assigned to established genera and associated novel or validly named species *.

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</tr>
<tr>
<td>S. atacamensis</td>
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<tr>
<td>S. bullii **</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>S. desertii</td>
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</tbody>
</table>

* Uncharacterised members of the phylum Actinobacteria (sensu Goodfellow, 2012) have been isolated from highly acidic habitats and permaforest soils.


* Filamentous actinobacteria

** Novel specialised metabolites produced by members of these species.
Table 2. Examples of fungi isolated from selected extreme biomes and assigned to established genera and associated novel or validly named species *.

<table>
<thead>
<tr>
<th>Atacama Desert soils</th>
<th>Permaforest soils</th>
<th>Deep-Sea sediments</th>
<th>Saline/hypersaline habitats</th>
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<tbody>
<tr>
<td>Genera</td>
<td>Genera</td>
<td>Genera</td>
<td>Genera</td>
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<td>Alternaria</td>
<td>Aspergillus</td>
<td>Aspergillus</td>
<td>Aspergillus</td>
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<td>Amandinea</td>
<td>Cladospora</td>
<td>Cryptococcus</td>
<td>Cladosporium</td>
</tr>
<tr>
<td>Aspergillus</td>
<td>Cladosporium</td>
<td>Pichia</td>
<td>Emericella</td>
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<td>Baellia</td>
<td>Geomyces</td>
<td>Graphium</td>
<td>Eurotium</td>
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<tr>
<td>Caloplaea</td>
<td>Oidiodendron</td>
<td>Penicillium</td>
<td>Horta</td>
</tr>
<tr>
<td>Cladosporium</td>
<td>Penicillium</td>
<td>Rhodotorula</td>
<td>Penicillium</td>
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<tr>
<td>Diploicia</td>
<td>Thielavia</td>
<td>Trimmatostruma</td>
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<tr>
<td>Fusarium</td>
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<td>Wallenia</td>
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<td>Myriospora</td>
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<tr>
<td>Neucatenulostroma</td>
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<td>Penicillium</td>
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<td>Rhizocarpon</td>
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<td>Ulocladium</td>
<td>Species**.**</td>
<td>Species**.**</td>
<td>Species**.**</td>
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<td>Am. efflorescens</td>
<td>A. ocraceo</td>
<td>A. westerdijkiae</td>
<td>H. werneckii</td>
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<tr>
<td>A. atacamensis</td>
<td>D. etaliformis</td>
<td>P. crustosum</td>
<td>T. salinum</td>
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<td>A. salisburgensis</td>
<td>O. truneatum</td>
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<td>D. canescens</td>
<td>P. crustosum</td>
<td></td>
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<td>M. smaragula</td>
<td>P. fumiculosum</td>
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<td>R. simillunum</td>
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</tbody>
</table>

* Little is known about fungi in highly acidic and hot springs habitats.


* Novel specialised metabolites produced by members of these species.

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** Extreme Habitats **

- Microbial Diversity
  - Selective isolation & colony selection
  - Dereplication of rare & novel isolates
  - Biosynthetic potential of strain libraries
  - Preparation of extracts of selected isolates
- Chemical diversity
  - Detection of novel compounds
  - Scale up of interesting new specialised metabolites
  - Novel structural families
  - Novel biological activities

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Fig. 1
Fig 2
Fig 6

Fig 7
Fig 8