Heliyon 9 (2023) e16723

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Review article

South Africa's indigenous microbial diversity for industrial applications: A review of the current status and opportunities

Varsha Chhiba [a], Priyen Pillay [a], Sibongile Mtimka [a,b], Ghaneshree Moonsamy [a], Lusisizwe Kwezi [a], Ofentse J. Pooe [b], Tsepo L. Tsekoa [a,*]

[a] Future Production: Chemicals Cluster, Council for Scientific and Industrial Research (CSIR), Pretoria, South Africa
[b] School of Life Sciences, Discipline of Biochemistry, University of KwaZulu-Natal, Durban, South Africa

ARTICLE INFO

Keywords:
Africa
Microbial diversity
Culture collection
Metagenomics
Metaviromics
Research networks
Biocatalysis
Industrial applications

ABSTRACT

The unique metagenomic, metaviromic libraries and indigenous micro diversity within Southern Africa have the potential for global beneficiation in academia and industry. Microorganisms that flourish at high temperatures, adverse pH conditions, and high salinity are likely to have enzyme systems that function efficiently under those conditions. These attributes afford researchers and industries alternative approaches that could replace existing chemical processes. Thus, a better understanding of African microbial/genetic diversity is crucial for the development of "greener" industries. A concerted drive to exploit the potential locked in biological resources has been previously seen with companies such as Diversa Incorporated and Verenium (Badische Anilin-und SodaFabrik-BASF) both building business models that pioneered the production of high-performance specialty enzymes for a variety of different industrial applications. The market potential and accompanying industry offerings have not been fully exploited in South Africa, nor in Africa at large. Utilization of the continent's indigenous microbial repositories could create long-lasting, sustainable growth in various production sectors, providing economic growth in resource-poor regions. By bolstering local manufacture of high-value bio-based products, scientific and engineering discoveries have the potential to generate new industries which in turn would provide employment avenues for many skilled and unskilled laborers. The positive implications of this could play a role in altering the face of business markets on the continent from costly import-driven markets to income-generating export markets. This review focuses on identifying microbially diverse areas located in South Africa while providing a profile for all associated microbial/genetically derived libraries in this country. A comprehensive list of all the relevant researchers and potential key players is presented, mapping out existing research networks for the facilitation of collaboration. The overall aim of this review is to facilitate a coordinated journey of exploration, one which will hopefully realize the value that South Africa's microbial diversity has to offer.

1. Introduction

Microbes are some of the most ubiquitous and abundant organisms that reside on planet Earth and play a crucial role in creating the

* Corresponding author. P.O. Box 395, Pretoria, 0001, South Africa.
E-mail address: TTsekoa@csir.co.za (T.L. Tsekoa).

https://doi.org/10.1016/j.heliyon.2023.e16723
Received 19 September 2022; Received in revised form 5 May 2023; Accepted 25 May 2023
Available online 1 June 2023

conditions needed for life. In this way, microbial ecosystems define the boundaries of the biosphere and perform functions that are vital for ecosystem development and the general health of other living beings. The effort to extract value from South African microbial biodiversity has been the subject of several explorative as well as functional studies [1–3]. The utility and economic value of indigenous microbial biodiversity is on the rise with academic research interest, together with industry needs analysis being the key drivers in this field. With advancements in genome sequencing, scientists are now able to characterise the wealth of microbial diversity in various environments ranging from the icy plains of Antarctica to the hyper-arid regions of the Karoo in Southern Africa. Furthermore, by using culture-independent processing methods, the uncultivated microbiota can now be studied and their unique biochemistries exploited [4–6]. Fig. 1 indicates the various regions of microbial diversity on the African continent recently studied.

Microbial and genetic diversity can also shape novel biocatalytic and green chemistry processes (Fig. 2), which play an important role in reducing the dependence on traditional, harsh synthetic chemical processes [7]. Projections show that biocatalysis may provide a superior organic solution over classical chemistry processes in 10% of processes [8,9]. Biocatalysis can also help create conducive conditions for expression workhorses to thrive and perform their desired functions. However, there are alternative molecular options that involve cloning of single genes or entire gene clusters for subsequent heterologous expression of secondary metabolites, further unlocking an organism's biosynthetic potential with a relatively small genetic footprint [7]. When combined with microbial anaerobic degradation, there is an opportunity for microbial diversity to address the inadequacies presented by non-renewable traditional energy sources that can deplete over time and are very costly to sustain [10].

The ability of microbes to manufacture molecules with relevant and diverse functionalities that advance key sectors, such as pharmaceuticals, advanced plastics, and composite materials, as well as the mining and manufacturing sectors are the tip of the proverbial iceberg. Novel biological processes that show atomic efficiency and reduced waste production, while eliminating the use of hazardous chemical synthetic processes, are required to demonstrate favourable techno-economics [11]. This review explores African microbial diversity with an emphasis on its commercial potential and summarizes the current knowledge relating to ongoing research conducted in South Africa, highlighting research and development networks that have been established.

2. Microbial diversity - life in the unlikeliest places on the african continent

The isolation of microbes from unusual and at times uninhabitable regions on our planet is a clear strategy followed by many when attempting to locate biological systems demonstrating extraordinary biochemical capabilities. At a continental level, the existence of eight recognized biodiversity hotspots in Africa makes the possibility of finding unique biochemical capabilities very likely. These identified hotspots include the Succulent Karoo area, the Cape Floristic Region, the Maputaland-Pondoland-Albany region, Madagascar and the Indian Ocean Islands, the Horn of Africa, the Guinean forests of West Africa, and finally the eastern Afromontane region. Although many of these regions have been highlighted for their diverse fauna and flora populations, not much information is

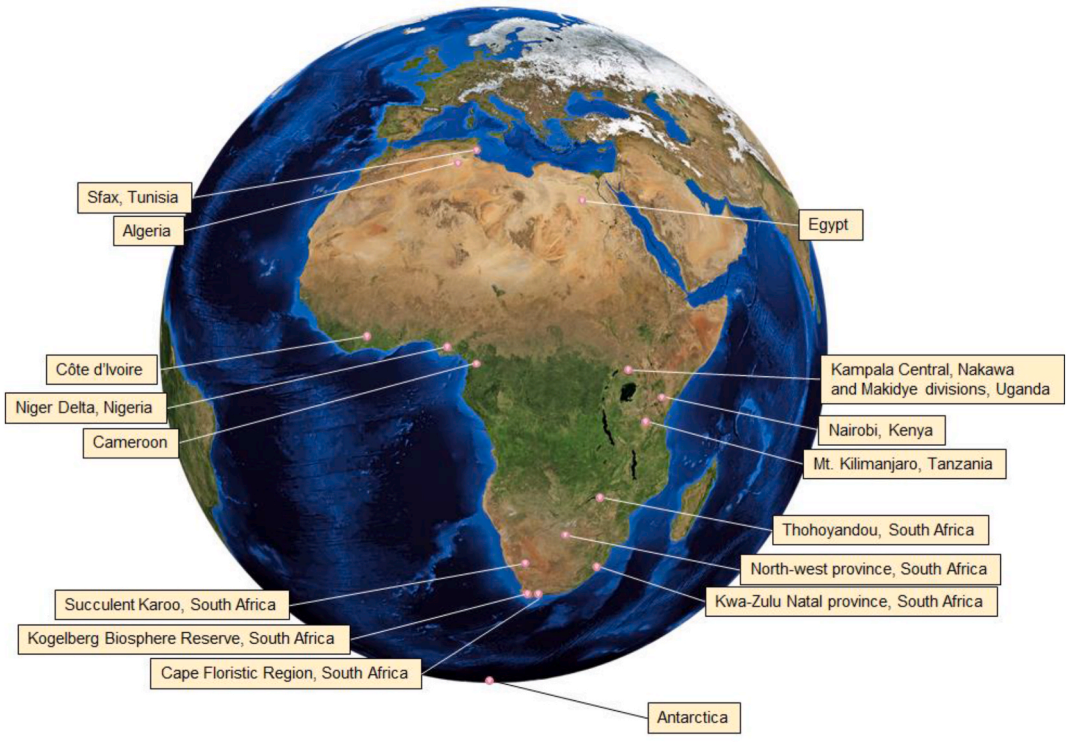

Fig. 1. A map of Africa highlighting key biodiversity hotspots located on the African continent (picture courtesy from pixabay).

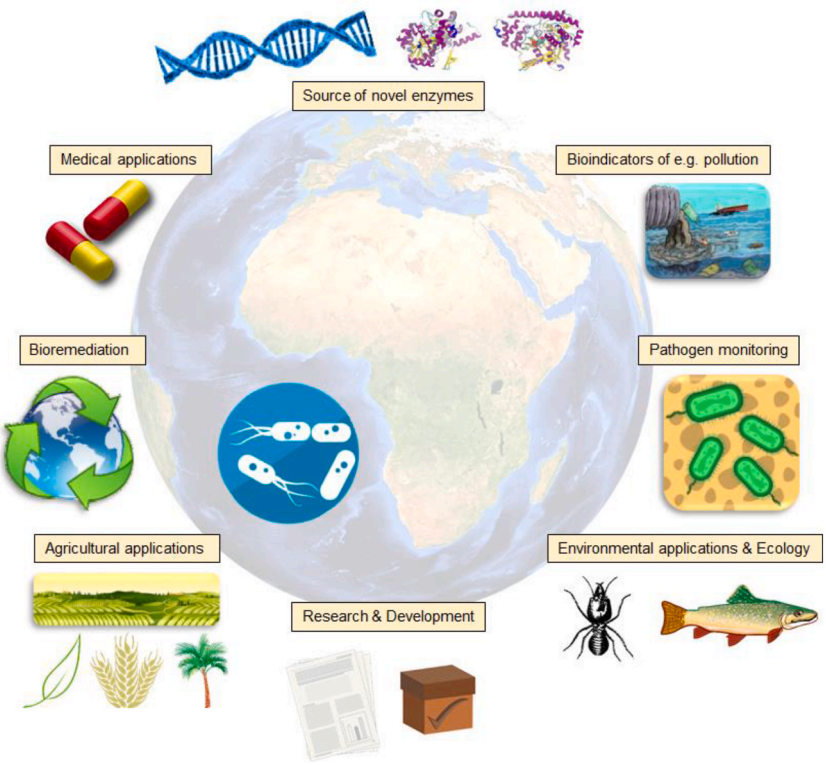

Fig. 2. Diagram showing the significant role microbes play in the industry and everyday lives.

available on microbial diversity profiles. This is an area of research that needs to be expanded in the future. However, the journey towards exploiting the microbial and genetic workhorses in South Africa is already underway, and the road to commercialization takes us from the pristine nature reserves of Kogelberg Nature Reserve [1] to the heavily polluted Olifants River catchment area in South Africa [12] with each unique biome teeming with novel microbes (Fig. 1). The main Southern African Research Institutions conducting research in these areas are listed below.

- ***Institute for Microbial Biotechnology and Metagenomics (IMBM):*** The commercial potential of the indigenous microbiota of Africa has been demonstrated by research conducted at the Institute of Microbial Biotechnology and Metagenomics (IMBM) at the University of the Western Cape (UWC, Western Cape Province). Research conducted under the directorship of Professor Marla Trindade-Tuffin (SARChi Research Chair in Microbial Genomics) has yielded considerable information relating to the commercial potential locked into local microbial and genetic libraries. One of their notable discoveries was a novel olsB gene, capable of producing ornithine lipid(s) that act as biosurfactants. This gene was extracted from a lake sediment soil sample from the Buffelspruit Lake area, Northwest province [13]. In addition, they have provided a snapshot of the antibacterial potential of marine sponges collected off the coast of Algoa Bay (Eastern Cape province) against the multidrug resistant strain *Escherichia coli* 1699 [14]. They have also isolated novel β-xylosidases from horse manure compost, finding enzymes that have greater thermostability under high concentrations of xylose [15].
- Under the banner of the TIA (Technology Innovation Agency - South Africa) and PharmaBio-funded LEAF project (Lignocellulosic Enzymes for Agricultural Feedstocks project), 90 cellulase enzymes, 36 *Endo*-β-1,4-xylanase enzymes, 6 Mannanase enzymes (2 provisional patents filed), 25 α-L-Arabinofuranosidase enzymes, 15 β-Glucosidase enzymes, and 203 unique lipase/esterase enzymes have been isolated and characterised at the IMBM. These microbial and genetic libraries assist both national and international businesses such as Loreal, Anglo-American, Evonik, Biodx, (South Africa), and Holiferm (Manchester UK), to name but a few. The coordinated research efforts of IMBM have yielded a significant footprint in the global biocatalytic market.
- ***The University of the Free State (Yeast Culture Collection):*** The University of the Free State (UFS) in the Free State province houses a unique yeast repository currently under the curatorship of Professor Carolina Pohl-Albertyn (sasm.org.za/culture-collections/60-yeast-culture-collection-university-of-the-free-state.html) (SARChi Research Chair for pathogenic yeasts). Being one of three nationally recognized South African National Biodiversity Core Biobanks, this yeast culture collection houses the largest collection of *Lipomyces* species in Africa. These strains are of interest due to their bioaccumulation of triacylglycerols, which find application as biodiesel intermediates. Furthermore, yeast strains stored in this repository can also be used for xylose production.
- ***Agricultural Research Council (ARC):*** Moving towards the administrative capital of South Africa, there lies one of the largest registered microbial repositories in Africa. Here, the Plant Pathogenic and Plant Protecting Bacteria Collection (PPPPB- Curator Dr.

Teresa Goszynska), the South African Rhizobium Culture Collection (SARCC), the South African National Collection of Fungi (SANCF), as well as the Vaccine Antisera Collection (VAC) store many different plant-associated bacterial, fungal and viral antisera collections (Tshwane, Gauteng province). Commercialized strains from these collections include those used to treat crown gall disease in grapevines (Department of Agriculture, Land Reform and Rural Development), as well as a variety of plant growth-promoting Rhizobium strains, studied in partnership with private companies such as Stimuplant, SoyGro, Nutrico, and Victus Bio.

- ***Centre for Microbial Ecology and Genomics (CMEG):*** The Centre for Microbial Ecology and Genomics (Director: Professor Don Cowan) based at the University of Pretoria houses one of the largest research teams in the Faculty of Natural and Agricultural Sciences of the University. Researchers from many countries collaborate under the CMEG research banner to study the effects that micro- and macroenvironment characteristics have on microbial community structures. Some of the studies conducted here include the evaluation of the molecular ecology of the Namib desert. The development of a desert soil metaviromic library has allowed researchers to assess the diversity of double-stranded bacteriophages (such as *Claudovirales* and *Mimiviridae*) at this unique environmental site [16]. Both culture-dependent & culture-independent methods were employed to further characterise virus-host networks.
- Further research areas of interest to researchers and funding bodies include marine ecology studies of the Southern Ocean investigating surface, middle, and deep-level water samples, and their corresponding microbial community structures. Additional work funded by USAID under the banner of the African Soil Microbiology Project has sought to better understand the role microbes play in creating healthy soil ecosystems.
- ***Council for Scientific and Industrial Research (CSIR):*** The CSIR campus located in Tshwane (Gauteng province) has collaborative research efforts with several academic institutions; initiating large-scale studies into the identification of novel nitrile-degrading microorganisms. Combined screening efforts between CSIR (Chemicals Cluster, Division 1), The University of Witwatersrand (Chemistry Department), Tshwane University of Technology (Department of Biotechnology and Food Technology), as well as the University of Cape Town (UCT: Aaron Klug Centre for Imaging and Analysis) identified a diverse library of Nocardiaforms such as *Rhodococcus rhodochrous* and *Rhodococcus erythropolis* capable of regioselective and enantioselective conversion of a variety of aliphatic, aryl-aliphatic and aromatic nitrile substrates [17,18]. The primary nitrile degradation pathways observed in these strains revealed the presence of cascade nitrile hydratase (NHase) and amidase enzyme systems, as well as the presence of inducible nitrilase enzyme expression systems [19].
- Other efforts of the CSIR include that of the Bioprocess Development group, which houses a database of approximately ~350 wild-type *Bacillus* organisms isolated from various niche environments. These organisms have been characterised for their ability to be used in custom-made biobased treatment technologies. To date, several technologies have been developed from concept to commercial scale, some of which have been licensed to local SMMEs, and other technology demonstrators are available for licensing by interested parties. The areas of application include the use of biobased products such as broiler feed probiotics [20–23], biological agents for use in aquaculture [24–28], use of microorganisms as soil stabilizers [29–32], consortium-based products for use in the remediation of hydrocarbons [31,33,34], bioremediation of wastewater [35] and the use of biological agents for the treatment of fats, oils and greases. Other areas of application include the use of biobased products in agriculture, the preparation of microbial starter cultures, and food preservation agents.

3. Cell culture collections and whole cell biotransformation

Culture collections and microbial repositories play a crucial role in conserving microbial resources while providing access to authentic biological material required by research institutions. Research institutions in turn apply microbes to biocatalytic process steps that are tailored toward generating industrially relevant Advanced Pharmaceutical Intermediates (APIs). In biotransformation reactions, cell growth (the phase required for enzyme production) and the production phase are separated, such that the substrate-to-product conversion utilizes resting cells. Meanwhile, fermentation processes see product generation coupled with cell growth. In essence, both processes allow for the execution of multistep reactions using cheap and abundantly available raw materials.

In South Africa, a government-driven initiative to support the preservation of local microbial biodiversity resulted in the generation of the South African National Core Biobanks (SANBI Biobanks) (http://www.bbsa.org.za). By policy, the SANBI Core Biobanks undertake to support the management of some cell culture repositories that include cell culture collections located at the IMBM (http://imbm.co.za), the ARC (https://www.arc.agric.za/arc-ppri/Pages/Biosystematics/Mycology%20Unit%20 (Fungi)/Mycology-National-Collection-of-Fungi.aspx), as well as the Yeast Culture Collection (https://sasm.org.za/culture-collections/60-yeast-culture-collection-university-of-the-free-state.html) located at the UFS. The core Biobanks in turn, provide open access to information from these culture collections and implement standards and procedures required to contribute towards achieving the objectives as set out by the BBSA. Professor Michelle Hamer functions as the Director of the National Science Collections Facility (NSCF) and is the lead for the BBSA. At its heart, the mission of the SANBI Biodiversity Biobanks is to document new species of bacteria that represent a unique genetic resource for the country. The Biobank also provides checklists and maintains databases or links to information on bacteria of economic importance to South Africa. They also create databases of relevant information and links to information that could guide identification. In addition, they seek to establish a South African systematic network that allows researchers to work together in areas of bacterial systematics and diversity and, finally, they promote research into the impact that bacteria have on biodiversity, conservation, and ecosystem functioning (bbsa.org.za).

4. The use of metagenomics and functional screening to analyse microbial diversity

With the increasing demand for greener processes, metagenomics bioprospecting is believed to be one of the most likely pathways to the discovery of candidate molecules required to serve the needs of industry [36]. Newer technologies and novel biological processes could boost intra-African trade flows while potentially developing technologies that could find application in other diverse economies [37]. The extent of this indigenous microbial diversity is largely derived from the 16 S rRNA sequencing analysis, which can reveal a number of different species of microbes, isolated from a single region of interest [38].

Functional selection is also an effective approach to the extraction and identification of candidates, and can be achieved in two ways: 1) by designing an assay that encourages colony growth [39], and 2) the appearance of clearing zones around individual colonies that indicate the hydrolysis of a preselected substrate for the target enzyme's substrate specificity [40].

4.1. Sample collection, storage and DNA extraction methods

In the past decade, minimal invasive sampling has been realized and applied more frequently. Mathur et al. (2004) report that accessing samples from the environments of diverse hotspots around the world is crucial, as this increases the likelihood of isolating novel genes and biocatalysts [41]. Each environment presents different challenges with metagenomic investigations requiring specifically designed methods accommodating for varied soil physico-chemical factors [42]. These challenges have given rise to molecular techniques that involve the extraction of environmental DNA (eDNA) from a selected environment; the extracted eDNA is then processed and cloned into an appropriate vector (either a plasmid, cosmid, fosmid, or bacterial artificial chromosomes, depending on the size of the clone) to create a metagenomic library [43]. The library can either be sequenced directly or functionally screened for the presence of any desired properties or traits encoded by genes or gene clusters harboured within the library based on the target. Genomic DNA from an environmental sample can also be extracted directly or indirectly. To acquire high purity and high quality DNA, it is crucial that the extraction approach be carried out adequately to access the entire microbial community [44]. In direct DNA extraction methods, cell sample lysis is carried out to retrieve genetic material from the sample; whereas, in the case of indirect extractions, cells are isolated from the environmental sample and then lysed, allowing extraction and characterisation of the host genetic material [45,46].

4.2. Advances in sequencing technologies, assembly, and annotation

It was once the exclusive domain of well-funded international consortia, today genome sequencing and *de novo* assembly have become increasingly affordable, making it possible for smaller research groups to participate and benefit from the great advantages these technologies offer. Third-generation long-read DNA sequencing technologies (e.g. PacBio Single Molecule, Real-Time (SMRT) Sequencing) are increasingly being used, providing access to extensive genomic toolkits for use in research. In metagenomics, generating a high-quality genome assembly/annotation has become an indispensable tool, allowing for a better understanding of the biology of any species. Jung et al. (2020) also outlined twelve steps to assist researchers in starting any genome-based sequencing project by presenting guidelines that are broadly applicable (to any species), sustainable, and cover most of all aspects of genome assembly and annotation projects from beginning to end [47].

4.3. Advances in functional metagenomics and culturing the unculturable

Earlier studies have shown that most environmental bacteria are very recalcitrant to cultivation [48]. The advent of metagenomics meant that the biotechnological potential of these bacteria could be accessed by cloning DNA sequences recovered from the environment itself. Once novel genes are discovered, the major downstream hurdle is the expression of the target molecule at sufficient levels to ensure that the process is techno-economically feasible [7]. The limitations associated with microbial cultivation spurred the development of culture-independent metagenomic strategies for gene discovery; thus allowing for whole microbial genome isolation from complex environmental samples [49].

Microbial diversity applications are increasingly finding use in bioremediation studies and agricultural processes and have also been adopted in biocatalytic processes to produce active APIs. This is not surprising since environmental microbes are extremely diverse and have a variety of metabolic activities and products that could have significant industrial applications [50]. The various applications of African microbial diversity studies undertaken are depicted in Fig. 2.

4.4. Microbial strains and metagenomic/metaviromic studies undertaken in Southern Africa

Climatic patterns vary considerably along the length and breadth of Southern Africa with Mediterranean-type weather conditions observed in the Cape Floristic Region giving way to the hyperarid conditions in the Namib desert. South Africa's rich mining history (gold, platinum and diamond mines) also provides unusual subterranean environments that have been extensively studied by researchers at the UFS. A glance at Supplementary Table 1 provides information about the metagenomic and metaviromic libraries prepared from these diverse South African environments. Soil virome assembly and viral community studies have been conducted in the Namib Desert by researchers at CMEG and the IMBM [51–54].

In another extreme environment, joint research by Princeton University and UFS researchers studied six thermal springs that represented topographically driven meteoric water located along fracture zones found in the Limpopo Province [55]. The average

temperature of these springs was found to range from 40 °C to 70 °C, with the water pH varying between pH 8–9. DNA sequencing (16s rRNA analysis) showed that the dominant phylum identified in these thermal springs was that of Proteobacteria. These unique microorganisms look poised to retain functionality under elevated temperature conditions, while potentially housing moderately alkalophilic enzyme systems.

Subterranean studies conducted at the Beatrix mine located in the Free State resulted in the preparation of an almost complete draft genome of the previously uncultured deep mine Archaeon *Candidatus bathyarchaeota* BE 326-BA-RLH [56]. This anaerobic, methanotrophic archaeon genome was found to encode proteins involved in hydrogenotrophic, acetoclastic methane metabolism as well as nitrate reduction. Earlier studies carried out by Abbai and colleagues at the same mine also prepared a metagenomic library containing a variety of genes from previously uncultured Archaea and bacterial species [57].

Within the Chemicals Cluster at the CSIR, researchers have created several unique bacterial and viral genomic libraries. Work by Segobola and colleagues resulted in the creation of a unique metaviromic library prepared from samples taken from the Koegelberg Biosphere Reserve (Western Cape). Taxonomic identification revealed the presence of members from the order *Claudovirales* [1]. The characterisation of this metaviromic library resulted in the identification of several useful DNA manipulating enzymes that have been commercialized subsequently through joint research license agreements with Capebio™ (https://capebiosa.com/).

The work carried out at the CSIR has also resulted in the preparation of three additional metagenomic libraries, namely the acidic leachate metagenomic library prepared from samples taken from a regional landfill site in Chloorkop, Gauteng Province [58], a metagenomic library prepared from genetic material collected from the hindgut of *Trinervitermes trinervoides* [59], as well as a metagenomic fosmid library prepared from rumen bacteria from bovine subjects [60].

In addition, phage therapy applications have shown potential for use as alternative treatment strategies against antibiotic-resistant bacteria, with some phages employed in epidemiological fingerprinting for rapid detection of pathogenic bacteria in samples. Researchers at the IMBM have created a unique metaviromic library of human phages. A total of 130 new phage species were included in this library, many of which were found to be present in several test individuals [61]. In this study, a bacteriophage related to the *Staphylococcus capitis* phage Stb20 was also found, which researchers speculate may play a commensal role in the regulation of pathogenic bacterial levels on the skin.

4.5. Biofuel Production and hydrocarbon degradation

Successful bioethanol production processes must be techno-economically feasible, that is, yielding more energy than that required for production. Cellulose binding and cellulase/xylanase synergy are important together with the identification of novel biocatalytic systems that improve existing ethanol production yields. Researchers at IMBM and CMEG documented the discovery of a pyruvate decarboxylase enzyme (PDC) from *Gluconobacter oxydans* that was found to convert pyruvate to ethanol via the intermediate acetaldehyde [62] (Supplementary Table 2). This enzyme showed a high affinity for pyruvate (120 μM at pH 5) with high catalytic activity and good *in vitro* thermostability (optimum temperature of 55 °C with 40% enzyme activity retained for 30 min at 65 °C). The heterologous expression of this protein in the thermophilic strain *Geobacillus thermoglucosidasius* resulted in the successful expression of PDC with bioethanol yields as high as 0.35 g/g of ethanol per gram of glucose spent.

When looking at hydrocarbon degradation, many microorganisms achieve this feat using aerobic or anaerobic reduction processes [63]. Approximately 175 prokaryotic genera containing several different phyla of Bacteria and Archaea, and an equivalent number of fungal genera have been found to use hydrocarbons as their sole carbon source during growth [64,65]. UWC researchers were successful in isolating an indigenous strain of *Trichoderma atroviride* ES-11 capable of degrading 82% of particulate coal (10 g/L) over a 21-day incubation period, showing a 50% reduction in as little as 6 days [66] (Supplementary Table 2). Low levels of glucose supplementation aided coal solubilization (5 g/L^{-1}), while higher glucose levels had an adverse effect (10 g/L^{-1}). Several intracellular enzymes involved in coal depolymerization were identified in their study, with a constitutive phenol hydroxylase enzyme observed. Other enzymes such as 1,2-dihydro-1,2-dihydroxy anthracene dehydrogenase, 2,3-dihydro biphenyl-2,3-diol dehydrogenase, 3, 4-dihydro phenanthrene-3,4-diol dehydrogenase, and 1,2-dihydro-1,2-dihydroxy naphthalene dehydrogenase were found to be activated in the presence of coal [66].

In the Free State province, the microbial isolation of gram-negative halophilic bacteria such as *Salinivibrio* sp. From Salt Pan ecosystems was followed by comprehensive metabolic fingerprinting. This enabled the tracking of the catabolic pathways native to these naphthalene-degrading microorganisms [67]. This *Salinivibrio* sp was found to grow under alkaline conditions degrading naphthalene to generate a battery of different intermediates. These included the production of 2,3 butanediol (a precursor in chemical production, antifreeze agent and intermediate used in synthetic rubber production), hexahydro-3 (2-methyl propyl)-pyrrolo [1,2a] pyrazine-1,4-dione (antifungal, antibacterial, antinematicidal, and anticancer agent), aziridine (potential therapeutic agent); and ethyl acetate (used in chemical processes and employed in the wine manufacturing industry).

4.6. Cellulase & xylanases

Cellulases, also known as endoglucanases, randomly hydrolyse β-1,4 bonds of cellulose, producing oligosaccharides, such as cellobiose and glucose [68]. These enzymes convert cellulosic materials into ethanol, organic solvents, and organic acids. They are also used to enhance the nutritional value of feed and are applied in the paper and pulp industry [69]. Xylanases can be used as an alternative “green” process for the bleaching of cellulose pulp in the pulp and paper industry. Accessory enzymes such as xylanases, laccases, and ligninases that may be present in the production process add to the production of high-quality cellulose pulp [70]. Rumen digestive tracts, similar to termite hindgut microorganisms, house microbes that hydrolyse plant biomass. A metagenomic library

comprising 70 000 fosmids was created from bacterial DNA isolated from bovine rumen samples. This library was subjected to functional screening to identify any cellulose hydrolysing enzyme activity [60] (Supplementary Table 3). Subsequently, two clones were identified through early qualitative activity screening (cel5A and cel5B) and subjected to further characterisation studies. Both encoded for the production of thermotolerant alkaliphilic endocellulase/xylanase enzymes capable of hydrolysing the β-1,4 bonds located in cellulose and hemicellulose polymers [60]. In addition to this work, other functional screening studies carried out on a termite hindgut metagenome library prepared at the CSIR revealed open reading frames for 25 cellulases and hemicellulase enzymes [71]. These enzymes were further classified and found to belong to 14 different glycoside hydrolase families. Eight of the 25 enzymes were cloned into *E. coli* expression systems and found to include four endocellulase enzymes, one exocellulase, two endoxylanase enzymes, and an alpha-fucosidase enzyme.

In other expression studies, three fosmid preparations from a high-temperature compost sample analysed at IMBM resulted in the identification of three alpha-arabinofurananosidase (AFase) enzymes by Fortune et al. (2019). Alpha-arabinofuranosidase enzymes are important debranching enzymes that remove arabinose from arabinoxylan and arabinoxylooligomers, thus promoting the hydrolysis of plant biomass samples. Both Afase E3 and H4 exhibited optimum activity at 60 °C, while Afase D3 showed optimum activity at 25 °C. An interesting observation made was that Afase D3 maintained enzyme activity at 90 °C. All three Afases showed maximum activity within a pH range of 4.0–6.0 [72]. Lastly, Selvarajan and colleagues showed that microbial isolations from a Free State Salt Pan biome yielded two cellulase-producing microorganisms. The microbial samples S7 and SP9 showed low levels of cellulase activity (1.95 U/ml) with an alkaline pH optimum observed in the case of both enzymes [67].

4.7. Lipase/esterase enzymes

Lipase and esterase enzymes find significant applications in the biotechnology sector. The key difference between the two enzymes is the substrate preference that each demonstrates, with lipase enzymes hydrolysing long-chain triacylglycerols to generate glycerol and fatty acids products, while esterase enzymes hydrolyse water-soluble short acyl chain esters [68]. Both esterases and lipases are widely used in industrial processes due to their regioselectivity, chemoselectivity, and enantioselectivity preferences and the stability that these enzymes demonstrate in organic solvent systems. Both lipase and esterase enzymes also find application in the pharmaceutical fine chemicals production industry (~90% purity) [68]. Studies carried out by several independent South African institutions have identified novel esterase enzymes from unique sampling sites. Supplementary Table 4 provides a recent snapshot of lipase/esterase published data over the last 2 decades from some of these institutions. Rashamuse et al. (2007) isolated several different esterase-producing microorganisms and created unique expression libraries at the CSIR. This resulted in the isolation of an of family VIII esterase-producing *Burkholderia multivorans* species (UWC10) that showed a preference for short-chain *para*-nitrophenyl and beta-naphthyl esters with no observable activity against beta-lactam substrates. The same microorganism was also found to encode a gene for a ferulic acid esterase (estEFH5) which post cloning showed a preference for short-chain nitrophenyl esters (C2 and C3) and ethyl ferulate substrates [73]. Several ferulic acid esterase enzymes were isolated from a leachate metagenomic library with translational analysis revealing the production of a thermolabile enzyme (Fae6) with a high affinity for methyl sinapate, methyl ferulate and ethyl ferulate. These suggest that this enzyme may be useful in a biorefinery processing environment [74]. An est22 gene located in the same leachate library showed sequence similarity to class VIII esterases, and the expressed protein showed preference for short-chain *para*-nitrophenyl esters (C2–C5). Est22 also hydrolysed some cephalosporin derivatives while leaving the amide bond on the β-lactam ring intact, making Est22 a possible candidate for use in the synthesis of modifications of cephalosporin-based molecules [2].

4.8. Nitrile-degrading enzymes (nitrile hydratases, nitrilases and amidases)

In general, nitrilase, nitrile hydratase (NHase), and amidase enzyme systems offer potential for use in the selective conversion of nitriles to their analogous acid or amide products. These are particularly useful in the biological synthesis of nonnatural amino acids and peptide therapeutic molecules (peptidomimetics). Nitrilases belong to the group of hydrolases (EC 3) that catalyse the hydration of nitrile compounds. Members of this family appear in plants, animals, and fungi [75]. Prokaryotes also appear to have phylogenetically related nitrilase enzymes present in them, indicating that the enzyme family may have appeared earlier in the separation of plants, animals, and fungi into their distinctive evolutionary families [76]. The larger nitrilase superfamily contains a group of closely related cyanide hydratase (CH) and cyanide dihydratase (CDH) enzymes, which hydrolyse cyanide to formamide, while the latter catalyses the conversion of nitriles to generate formic acid and ammonia. Nitrile hydratase enzymes, on the other hand, are metalloenzymes that catalyse the hydration of nitriles to their structurally related carboxamide products. They contain one of two different metal centers, a low-spin non-corrinoid cobalt [77–79] or a mononuclear non-heme iron [77,80] in their respective catalytic sites.

Many *Rhodococcal* species are of significant interest in the field of nitrile catalysis with a wide cell distribution of these strains observed in various soil samples [19]. *Rhodococcal* cells have found commercial success, and third-generation *Rhodococcus rhodochrous* strain J-1 cells are being used in the large-scale acrylamide production process [81]. This process was initially patented in 1988 (US patent number 5334519 A, Assignee Mitsubishi Rayon Co. Ltd.) with a similar strain that was isolated in Modderfontein, South Africa (*Rhodococcus rhodochrous* ATCC BAA-870) found to demonstrate enantioselective hydrolysis toward some classes of beta-substituted nitrile compounds [17,18]. These intermediates are important representatives of diverse pharmaceuticals such as beta-blockers, statins, and peptidomimetic compounds.

An amidase isolated from the strain *Bacillus pallidus* RAPc8 (UWC) was found to belong to branch 2 of the nitrilase superfamily (Supplementary Table 5). This thermophilic bacterium was found to exhibit favourable thermotolerance, with a 5-h half-life observed when tested at 50 °C and 60 °C. When the temperature was raised to 70 °C and 80 °C, the thermal half-life was reduced to 43 min and

10 min, respectively, with the resultant production of enantiomerically pure carboxylic acid products [82].

4.9. Novel enzymes and extremozymes

Life continues to thrive in environments that may be considered challenging (high temperatures & pressure, high salinity, low levels of available water, radiation, elevated or low pH conditions, or in the presence of high levels of heavy metals) [68]. Organisms with simple structures have been found to propagate well under extreme conditions as opposed to those housing complicated structures [83]. Extremophiles have gained a great deal of interest with thermophiles being some of the first microorganisms to be discovered [84]. Extremozymes (enzymes isolated from extremophiles) have molecular mechanisms for adaptation to extreme physicochemical conditions [84]. Three of these unique adaptations include: (a) excluding the factor by specific structural elements, (b) "detoxifying" the factor, and (c) living with the factor; the last mechanism reflected by the metabolic characteristics of a given organism [83]. Extremozymes are crucial for industrial applications due to their stability and/or bioactivity under these extreme conditions [84]. Some heat-resistant biomolecules have found application in the health sector. Extreme environments are expected to produce unique microbial diversity and unknown cellular gene products with attractive properties and new catalytic activities [68,84].

Due to the rise of multiple drug-resistant microorganisms, significant research has been focused on identifying novel bioactive compounds. Studies carried out at the IMBM sought to explore the rich diversity that exists in South African marine sponges (*Isodictya compressa* and *Higginsia bidentifera*) [14] (Table 1). This study produced 35 isolates that demonstrate antibacterial activity against the multidrug-resistant strain *Escherichia coli* 1699. Genome sequencing studies have also revealed the existence of novel biosynthetic

Table 1
Summary of studies conducted on indigenous extremophiles and their novel biocatalytic activity profiles. Institution data, associated research networks, and reference data.

Primary & Secondary Collaborating Institutions	Summary	Associated Publications
Professor IM Tuffin (IMBM) Professor Don Cowan (CMEG)	The molecular diversity of eubacteria was investigated in this study. Its specific foci were on the actinobacteria in hot springs located in Kenya, Zambia, China, and New Zealand. The biogeography of South African hot springs was also elucidated using the 16 S rRNA gene libraries. In summary, 28 major actinobacterial OTUs were found in this investigation.	[85]
	Within this investigation, open soil and hypolithic microbial communities along an East–West transect within the Namib desert were characterised by an inverse fog-rainfall gradient. Different microbial communities occurred in soil and in hypolithic zones structurally displaying a fog-related distribution.	[86]
	Three biological sand filters (BSF) were found to be contaminated with acid mine drainage (AMD) in this study. The remediation capacity and the evolution of autochthonous bacterial communities were assessed. The main processes found to be contributing towards AMD neutralization were microbial iron reduction and sulfate reduction associated with iron precipitation.	[87]
	In this study, the diversity of Ammonia-oxidizing bacteria (AOB) in the plant canopy of three South African fynbos-specific plant species was evaluated. Results confirmed that plant-species specific and monophyletic AOB clades were indeed present within fynbos canopy soils.	[88]
	Utilizing a combination of multiple displacement amplification of metaviromic DNA and deep sequencing approaches, viral communities of two diverse salt pans situated in the Namib Desert, Hosabes and Eisfeld, were examined. Many contigs belonging to the subfamily Gokushovirinae were discovered to be common in both environmental datasets. The salt pan metaviromes were observed to be unique and most strongly related to each other.	[16]
Professor IM Tuffin (IMBM)	Desert hypolithic communities were assessed within the Namib desert. Terminal restriction fragment length polymorphism (T-RFLP) assessment was utilized to evaluate the community structure of hypoliths and the surrounding soil environment. Hypolithic communities were found to be primarily inhabited by cyanobacteria affiliated with Pleurocapsales, whereas actinobacteria were found to be more widespread in the soil.	[89]
	The bacterial diversity associated with five South African marine sponges was investigated with two marine sponges, *Isodictya compressa* and *Higginsia bidentifera,* further selected to culture bacteria for antibacterial activity. Thirty-five isolates were found to exhibit antibacterial activity, and twelve were against the multi-drug resistant *Escherichia coli* 1699.	[14]
Dr. Oliver Zablocki (Ohio State University) Professor IM Tuffin (IMBM)	Provided within this study is a comprehensive taxonomic overview of phages that have been isolated precisely from terrestrial hot springs from around the world. Six thermophilic phage-related clades were identified. Analyses of the whole proteome showed clustering between phages that infect distinct host phyla, such as Firmicutes and Deinococcus–Thermus.	[90]
Dr. Slobodkina (Winogradsky Institute of Microbiology, Russia) Dr Esta van Heerden (iWater)	Two novel strains of thermophilic planctomycetes were discovered in terrestrial and subterranean habitats. The first Strain R1T was extracted from a hot spring (Kunashir Island, Russia) and the second strain SBP2T was extracted from a deep gold mine (South Africa). Both strains were capable of anaerobic respiration with nitrate and nitrite acting as electron acceptors as well as key drivers of microaerobic growth.	[91]
	A unique aerotolerant anaerobic, that is a moderately thermophilic, organotrophic bacterium, strain MBL-TLPT, was isolated from a sample of the microbial mat, in the TauTona gold mine, South Africa. The isolate was found to have the capability to be able to ferment yeast extract and mono-, oligo- and polysaccharides, including xanthan gum and starch.	[92]

pathways in these strains; this is good news for any biosynthetic process requiring a novel biocatalytic process.

A taxonomic overview of isolated phages and actinobacteria from terrestrial hot springs showed the presence of several unique microbial community structures in these environments [90,85]. In the review published by Zablocki et al. (2018), various thermophilic phages located in different terrestrial hot spring samples were subjected to evolutionary pattern analysis. Six distinct thermophilic phage-related clades were identified, with complete proteome analysis showing clustering between phages that infected different host phyla such as *Firmicutes* and *Deinococcus-Thermus* [90]. Meanwhile, actinobacteria populations isolated from hot springs in Zambia, China, New Zealand and Kenya showed that the temperature and pH values of individual samples ranged between 44.5 and 86.5 °C and pH 5–10 (research done at IMBM). Multivariant analysis indicated that the composition of the actinobacterial community was largely dependent on the temperature and pH profiles of the individual biomes [85].

Desert hypoliths are photosynthetic microbial assemblies that are found beneath translucent rocks. Research done at CMEG and IMBM has sought to characterise indicator species in Namib desert biomes with hypolithic communities showing a dominance of cyanobacteria affiliated with *Pleurosapsales*, while desert soil samples showed a dominance of actinobacteria instead [89]. IMBM researchers have mirrored these observations in an independent study conducted in their laboratories with many deterministic factors (interspecies interrelations and environmental factors such as temperature, pH, and relative humidity) and to a lesser extent factors such as relative growth rates of individual species in communities found to play a significant role in hypolithic & soil environments in the Namib desert [86].

Acid mine drainage (AMD) is a major concern for many people living in South Africa. Being a largely resource-driven economy with the first commercial mining initiated in the 1800s, environmental destruction over hundreds of years has resulted in high levels of synthetic iron and sulfate-rich acid streams identified in runoff and groundwater reserves. A study by Welz et al. (2014) at the IMBM examined potential biocatalytic remediation routes to treat AMD-polluted water [93]. A closely related microbial strain to *Clostridium beijerincki* was found to remediate AMD samples with an 81.5% reduction in Fe (II) levels, a 95.8% reduction in Fe (III) levels, and a 32.8% reduction in sulfate levels [87].

Ammonia oxidizing bacteria (AOB) play a vital role in global nitrogen recycling and nitrogen removal from wastewater plants. This is achieved through the oxidation of ammonia in a two-step process, which sees a nitrite product being formed via a hydroxylamine intermediate. The oxidation process results in the net gain of two electrons which is the source of energy for these bacteria [94]. In the publication by Ramond and colleagues (IMBM), the variety of AOBs in the plant canopy of *Leucadendron xanthoconus, Leucospermum truncatalum* and *Leucadendron microcephalum* was studied [88]. Through the creation of an amoA gene clone library, plant-specific AOB clades were identified in the canopy soil that had been studied.

Finally, deep-mine biomes offer unique deterministic factors that drive the formation of unusual microbial communities. Water samples from the Tautona gold mine and the Beatrix gold mines in the Free State have resulted in the identification of two thermophilic microorganisms, namely *Tepibacillus infernus* and *Thermogutta hypogae* [91,92]. *Tepibacillus infernus* was found to be a novel aerotolerant, anaerobic, organotrophic bacterium capable of growth at 25–58 °C, at pHs ranging from 5.6 to 8.8 and in the presence of up to 85 g/L sodium chloride. *Thermogutta hypogea* was found to grow under anaerobic conditions at temperatures ranging from 30 to 60 °C, and at a pH range of 7.5–8.0. Once again osmo-tolerance was observed with cells showing growth in media containing up to 30 g/L sodium chloride. This strain also uses simple sugars as electron donors and employs nitrate, nitrite, or elemental sulphur as terminal electron acceptor molecules. The significance of aerotolerant microorganisms such as *T. infernus* comes from a better understanding of their ability to grow in the presence of oxygen even though they do not require oxygen for ATP production. This knowledge may

Table 2
Summary of studies conducted on indigenous microorganisms demonstrating metal bioaccumulation capabilities. Institution data, associated research networks, and reference data.

Primary and Secondary Collaborating Institutions	Summary	Associated Publications
Professor Carolina Pohl-Albertyn (UFS) Dr Esta van Heerden (iWater)	Researchers at UFS studied the potential of a biopolymeric flocculant generated by a Terrabacter sp. Isolated from Sterkfontein Dam (Gauteng province) for application in aiding the aggregation of suspended solutes. Taxonomic evaluation via 16 S rDNA analysis revealed a bacterium demonstrating 98% similarity to Terrabacter species. Application of the purified bioflocculant to dairy wastewater effluents resulted in a reduction of both chemical and biological oxygen demand (C/BOD), suspended solids (SS), and water turbidity.	[104]
	Researchers at UFS investigated the bioaccumulation of europium (Eu) by a thermophilic bacterium, *Thermus scotoductus* SA-01. Furthermore, researchers found that the bacterium accumulated Eu, suggesting that *T. scotoductus* SA-01 could be potentially useful for the bio-recovery of rare earth metals from geothermal fluids.	[102]
Professor Derek Litthauer (UFS) Dr Esta van Heerden (iWater)	Researchers at UFS examined the effect of uranium on the growth of the bacterium *Thermus scotoductus* strain SA-01 as well as the whole cell U(VI) reduction capabilities native to the organism. Transmission Electron Microscopy observation on cells exposed to uranium showed extracellular and membrane-bound accumulation of uranium, suggesting the presence of a uranium metabolic pathway.	[103]
Dr. Sarah Staniland (University of Sheffield) Professor IM Tuffin (IMBM)	A biogeochemical study undertaken of a polluted wetland site in Kitwe, Zambia was conducted. From this study, a bacterium (*Comomonas testosteroni),* was isolated displaying resistance to, and intracellular cobalt accumulation. Further gene sequence analysis revealed the presence of heavy-metal resistance genes, implying the potential use of this organism for *in situ* treatment of contaminated aquatic systems.	[101]

provide us with a better understanding of the absolute requirements needed for life to exist.

4.10. Metal sequestration by microorganisms

Many metal processing industries, such as those involved in mining, petroleum processing, and within the automotive manufacturing industry, use processes that release a barrage of different heavy metals into the environment. These include metals such as copper, nickel, chromium, and iron, which pose a considerable risk to human and animal health and the environment [95]. Physiological processes such as metal oxidation and or reduction, chemical precipitation [96], filtration, liquid evaporation, electrochemical treatments, as well as reverse osmosis are some of the methods used to remove toxic heavy metals from industrial wastewater sources [97,98]. However, high reagent requirements, the generation of toxic sludges, as well as the unpredictability observed when attempting metal removal from material streams, make these processes problematic [99]. This, coupled with the lack of suitability for use when working with large-volume effluents, tends to draw attention towards finding alternate environmental remediation strategies. A long-term biological remediation strategy would work better, addressing each of these inherent issues while providing a long-term treatment strategy. Enhanced aggregation and biofilm production have been observed in many different bacterial and fungal cells exposed to metal stress. These changes in cell morphology appear to be triggered by cell surface alterations, which mediate the production of exopolymers that help reduce metal toxicity effects on the cells [100]. Local isolates showing metal accumulation include *Terrabacter* species, *Thermus scotoductus* strain SA-01, as well as *Comomonas testosteroni* species that have been studied by researchers at the UFS and IMBM. All of these strains have been found to facilitate metal bioaccumulation within their cell walls [101–104] while some show the presence of effective efflux pumps that allow metal removal from the cell [101] (Table 2).

Thermus scotoductus SA-01 cells were found to interact with europium at elevated temperature, with europium levels up to 1 mM in solution tolerated well [102]. However, concentrations of 2 mM resulted in inhibition of cell growth with tolerance for europium at lower concentrations, possibly due to metal biosorption, bioaccumulation as well as biomineralization [102]. The bioaccumulation of europium in these cells was observed on the cell surface with some precipitates observed intracellularly. Changes in the cell surface indicate that the metal is likely to interact with cell surface receptors, such as *S*-layer proteins and organic molecules. The presence of metal precipitates in the cells the authors postulate may be possible via the action of ABC transporter proteins [103,105]. The same *Thermus scotoductus* SA-01 cells were also found to reduce uranium (VI) to uranium (IV) under anaerobic growth cultivation conditions with lactate employed as an electron donor (1.25 mM concentration). Once again, a native ABC transporter protein was considered to play a role in this bioconversion process [103].

Comomonas testosteroni cells isolated from a heavy metal contaminated site in the Zambian Copperbelt region showed resistance to cobalt, bioaccumulation of metals and growth in the presence of 200 μM of the abovementioned metal [101]. Complete inhibition of cell growth was observed at 4 mM cobalt concentration, with cell tolerance to metals such as iron (up to 1 mM), manganese (1–2 mM) and nickel (500 μM) observed [101].

Finally, *Terrabacter* cells isolated from the Sterkfontein dam were found to produce a bioflocculant with considerably better flocculating efficiency than conventionally used poly aluminium chloride, polyethylenime, and alum [104]. The microbial flocculating agent demonstrated removal of aluminium (77.7%), manganese (74.8%), zinc (61.9%) and iron (57.6%) from dairy wastewater that had been processed.

4.11. DNA manipulating enzymes

DNA-manipulating enzymes can be classified into six broad categories based on the structure and function exhibited by these enzymes. These categories include polymerases, nucleases, ligases, end-modification enzymes, as well as topoisomerases [106]. Polymerase enzymes synthesize new polynucleotides that correspond to an existing DNA or RNA template. Rothwell and colleagues classified polymerases into seven classes: viz. A, B, C, D, X, Y, and RT [107]. Ligases are demarcated into two classes based on their cofactor dependency (NAD + or ATP). They function to join breaks in the nucleic acid backbone by creating phosphodiester bonds between opposing hydroxyl and phosphate ends [108]. Topoisomerase enzymes play a pivotal role in DNA replication, transcription, chromosome segregation, and recombination [109,110]. So far, five have been characterised and reported DNA topoisomerases have been described [109]. End-modification enzymes work by altering the ends of DNA molecules by the addition or removal of a phosphate groups. These enzymes differ from DNA polymerases, not requiring a template to synthesize new DNA [106]. Nuclease enzymes disrupt nucleic acid backbones by cleaving the phosphodiester bonds that bind individual units. Nucleases are classified into three subgroups, namely: 1) endonucleases (cleave internally located nucleic acids & play a role in the repair of DNA); 2) exonucleases (digest nucleic acids from the 5′ end or the 3′ end); and lastly; 3) endo-exonucleases that cleave DNA and/or RNA from both internal and terminal positions [110]. DNA-manipulation enzymes with their various applications play a crucial role in the fields of molecular biology and biotechnology [39]. New developments and research within this market have resulted in steady market growth that is projected to grow at a compound annual growth rate (CAGR) of 7.41% in the next five years [111]. Advances in DNA manipulation and diagnostic enzymes are believed to be key drivers of growth in both the research and biotechnology sectors. New technologies and techniques are constantly needed to facilitate the rapid discovery, expression, and characterisation of genes from local diversity.

The discovery of novel DNA manipulation technologies has been lacking and more attention is being placed on improving existing technologies. Several research institutes have been exploring the genetic biodiversity within South Africa to discover novel DNA-manipulating enzymes. The continuous interest in recombinant DNA technology, diagnostics, and gene editing continues to be a key driver towards the identification of novel products that can add value in these developing research areas. A novel approach to discovering some of these enzymes has been described by Mtimka et al. (2019) using applied metagenomics. This technique can be

further explored and advanced to discover more enzymes using various substrates [39]. Table 3 introduces a limited list of works that have been conducted in this field of research. A study conducted by Segobola et al. (2018) reveals the potential to valorise diversity hotspots in the discovery of novel enzymes, demonstrating the functional activity of some unique nucleic manipulating enzymes [112]. Furthermore, the sequence-based screening approach can also be used to discover novel enzymes and the success of that approach can also be seen in studies conducted at the Vaal University of Technology and UP [112,113].

5. Gaps and perspectives

Need for a national/regional biofoundry - The biofoundry concept is based on a four-pillar process: design, build, test, and learn [114]. Through the implementation of this testing and learning process, cell factories can be developed. South Africa, with its diverse microbial and genetic libraries, could house many unique biocatalysts that would be easily identifiable through the development of an integrated and accessible biofoundry. Biofoundry's would allow researchers and stakeholders access to potential biotechnological solutions that could help fast track greener biological processes.

Addressing regulatory and financial hurdles - Although efforts have been made to characterise and exploit South Africa's unique microbial repositories, this endeavour has faced numerous challenges within the biomanufacturing research sector. Competition with established incumbents with more lucrative economies of scale, regulatory complexities, together with the need for considerable capital input make business development out of reach for many small to medium enterprises (SMMEs). This, coupled with the lack of working capital needed for the first 2- to 3-year company development cycle, makes local start-up success numbers exceedingly low. Government intervention in this regard is vital. Adoption of financial support programs that provide research subsidisation and commercialization support can be a way to attract more interest from the private sector. Adopting tax reform policies for small-medium enterprises can also provide a vital lifeline to fledgling research industries. Two areas that could potentially derail the uptake of biological production processes are the presence of heavily regulated legislation around research, as well as the considerable cost to the industry when adopting newer processes. These are not isolated concerns, as many other countries adopting "greener" production processes have encountered similar problems. However, it is the adoption of creative counter approaches, such as relaxing existing legislation to favour industry uptake as well as government-approved tax exemptions for participating research and production industries, that has worked to entice industry towards adopting novel biologically driven production processes. Lastly, regulatory hurdles and slow legal processes make the product's time to market a lengthy and arduous process. Speedy and cost-effective processes are essential for the success of these business ventures.

6. Conclusions and future prospects

Microbial diversity has the potential to be Africa's richest source of biodiversity that creates vibrant local and sustainable economies. This current review has highlighted the wealth of microbial diversity and its diaspora within South Africa and other areas within the African continent. Microbial communities play a vital role in the functioning of all ecosystems; however, most microorganisms remain uncultivated, and their roles in natural systems are also unclear. Exploring indigenous microbial diversity has played a significant role in several industries, including agriculture, medicine, and bioremediation, and with the advent of newer and advanced sequencing technologies, a greater understanding of the environment can be achieved with the accompanying potential to add value to local industries on the continent. The study of microbial evolution and ecology has in fact been revolutionized by DNA sequencing and bioinformatic analysis and is expected to be further developed through culture-independent recovery methods of microbial genomes from environmental samples, which is a significant advancement in the study of natural African microbial communities. Early public

Table 3
Summary of studies conducted on indigenous metagenomic and metaviromic libraries showing DNA manipulating enzymes. Institution data, associated research networks, and reference data.

Primary and Secondary Collaborating Institutions	Summary	Associated Publications
Dr Tsepo Tsekoa (CSIR) Professor Don Cowan (CMEG)	CSIR and CMEG researchers studied the application of bioinformatics tools such as MetaVir and MG-RAST. This revealed Caudovirales, specifically the family *Siphoviridae*, as dominant in the soil samples and other associated viromes. Phylogenetic analysis of DNA polymerase classes B and B2, including *PolB*, *PolB2*, *terL,* and phage-related *T7,* demonstrated that many viral sequences are closely related to the order *Caudovirales.*	[112]
Dr Tsepo Tsekoa (CSIR) Professor Samantha Gildenhuys (UNISA)	Endonuclease genes were identified (and subsequently expressed) from a fosmid library extracted from the Koegelberg National Reserve. A total of 113 novel open reading frames (ORFs) encoding putative endonuclease genes and ORFs of unknown identity were identified. One of these endonucleases, designated Endo 52, was recombinantly produced with lambda DNA cleavage used to confirm functional activity.	[39]
Dr Naser A Feto (VUT) Dr Konanani Rashamuse (DSI)	Researchers at VUT employed a metagenomics approach to identify DNA-manipulating gene sequences from three hot springs located in Limpopo Province. Sequencing via Illumina MiSeq Next Generation Sequencing (NGS) resulted in 5 681 662 reads and 7338 contigs being identified. A fosmid library (containing approximately 2.16×10^3 clones) was constructed and distinct genes were identified. DNA polymerase, DNA ligase, and endonuclease II enzymes were expressed, purified, and characterised from this library.	[1,113]

participation will preempt concerns over these brand new technologies and create the demand that the industry requires to foster economic growth and success [115]. Whole cell enumeration, together with metagenomics, could provide an avenue to unlocking newer 'greener' bioprocess technologies. We have also highlighted the diversity of indigenous microorganisms, metagenomic, and metaviromic libraries located in South Africa, including three of the SANBI-recognized core Biobanks, and delved deeper into these libraries illustrating the potential locked up in these microbial stores. Each institution listed, from academia all the way through to research translation institutions, has made significant shifts towards commercializing their unique research capabilities. Centre stage in their endeavour is the identification of rapid, creative solutions and unusual technologies that fit the bill and do so elegantly, economically, and sustainably. Future African industries can then tap into the novelty of new biotechnology applications that arise from the discovery of novel enzymes and, in turn, foster the demand for such industrially relevant enzymes and processes.

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

Data availability statement

No data was used for the research described in the article.

Additional information

No additional information is available for this paper.

Funding

Funding for the preparation of this manuscript was generously provided to the CSIR by the Technology Innovation Agency (TIA), as well as the Department of Science and Innovation (DSI) Bioinnovations funding.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

A thank you is extended to all members and participants in the CSIR/DSI-funded inaugural Indigenous Microbiology for Industrial Applications workshop that was hosted virtually on the November 9, 2021.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e16723.

Supplementary Table 1
Summary of studies conducted on indigenous metagenomic and metaviromic libraries and their respective isolation regions

Primary and Secondary Collaborating Institutions	Summary	Associated Publications
Dr Tsepo Tsekoa (CSIR) Dr Konanani Rashamuse (DSI)	A leachate-fosmid library prepared by researchers at the CSIR resulted in the identification of a novel family VIII esterase (EstC). Biochemical characterisation revealed the enzyme's novel β-lactamase activity against the substrate Nitrocefin and showed its activity against the tertiary alcohol linalyl acetate.	[58]
	Trinervitermes trinervoides species collected from the Komatipoort area (Mpumalanga province) were subjected to hindgut symbiont isolations. The metagenomic libraries prepared from these symbionts were screened for FAE activity which resulted in the isolation of seven novel FAE proteins.	[116]
	A metagenomic library of approximately 70 000 fosmids was created from bacterial DNA that had been extracted from the bovine rumen (undisclosed abattoir located in Johannesburg, Gauteng province). Two clones from this study were further characterised revealing both endo-cellulase and xylanase activity.	[60]
	Viral DNA isolated from the Koegelberg Biosphere Reserve was used to prepare a metaviromic library (Western Cape province). Taxonomic, phylogenetic, and biochemical characterisation of this library revealed the presence of several members from the order *Caudoviral*es (more specifically the family *Siphoviridae*). Novel phage gene sequences were identified through this study.	[1]

(*continued on next page*)

Supplementary Table 1 (*continued*)

Primary and Secondary Collaborating Institutions	Summary	Associated Publications
Professor IM Tuffin (IMBM)	A metaviromic library prepared from soil samples extracted at a copper-rich site in the Namib desert was evaluated using a CLC-Workbench assembler. Taxonomic identification of genes located in the library revealed the presence of Circo-like proteins which are often linked to marine environments. Viral carryover inland from the Atlantic Ocean is proposed as a likely mechanism for the transport of these viruses	[52]
	A metaviromic library was prepared from skin sample microbes isolated from different individuals. Data obtained revealed 130 new skin phage species with similar phage population profiles found in different individuals. The study also revealed the presence of a potential commensal bacteriophage related to *Staphylococcus capitis* Stb20. This phage in the researcher's postulate could play a role in reducing pathogenic cell loads on the skin.	[61]
	Olonade and colleagues describe the isolation and detection of a prophage (Smhb1) capable of infecting *Salinivibrio kushneri* BNH (isolated from the Namib desert). Genome analysis revealed that the phage showed the most similarity to P2-like phages that are known to infect *Vibrio* species, however, they showed no relatedness to previously described *Salinivibrio*-infecting phages.	[117]
Dr. Oliver Zablocki (Ohio State University) Professor IM Tuffin (IMBM)	Researchers at IMBM sought to survey the diversity of viral communities present in the Brandvlei hot springs (Northern Cape Province). Transmission Electron Microscopy revealed varied viral morphotypes, including *Caudovirales*, lemon-shaped virions (*Fuselloviridae*-like; Salteroprovirus-like), and pleiomorphic virus-like particles. Metaviromic analysis further substantiated the presence of His1-like viruses. This study showed that moderately thermophilic aquatic environments can house highly novel viruses.	[118]
	Four soil metaviromes were prepared from soil samples taken at 30 km intervals extending from Walvis Bay towards Windhoek. Sequencing of the extracted DNA produced 93 519 306 reads with a mean read length of 142.5 bps. *Mycobacterium* phages were found to be the top sequence hit in all soils analysed. The second most identified phage isolates were *Rhodococcal* phages, followed by *Bacillus* phages and *Geobacillus* phages.	[119]
	A comprehensive taxonomic overview of phages isolated from various terrestrial hot springs around the world is provided in this journal. Six thermophilic phage-related clades were identified. Whole proteome analyses revealed clustering between phages that infect distinct host phyla, such as Firmicutes and Deinococcus–Thermus.	[90]
Professor Don Cowan (CMEG) Professor IM Tuffin (IMBM)	Researchers studied the viral communities associated with hypolithic microbial communities in the Namib desert. Research findings revealed that most bacteriophages located in this environment belonged to the order *Caudovirales* (more specifically the family *Siphoviridae*). An assessment of the metaviromic datasets prepared to show a high prevalence of cell wall degrading enzymes, phage-associated genes, and a unique class distribution of ribonucleotide reductase genes were located.	[51]
University of the Free State Professor Derek Litthauer	Biofilm collected from the Beatrix mine (located in the Free State Province) was used to create a metagenomic library containing material from various uncultured bacteria and archaea. The library was then screened for esterase activity with two enzymes, namely a phospholipase patatin protein and an isochorismatase family protein being identified. Both enzymes were sufficiently different from known esterase enzymes suggesting that subterranean environments may house unique biocatalytic agents/enzymes.	[57]
Dr Cara Magnabosco (Princeton University) Dr Esta van Heerden (iWater)	Researchers at Princeton University studied the microbial diversities in thermal spring samples (Limpopo province) versus deep fracture water found in South African mines (depth ranging from 1 to 3.1 km). Macro-driving characteristics such as anion and cation profiles in the 6 thermal springs and 6 deep fracture water were similar however the microbial communities in the thermal spring samples were very distinct, showing low similarity to the subterranean samples evaluated. All 12 environmental samples showed Proteobacteria dominance with only one genera *Rheinheimera* identified in all samples.	[55]
Dr Rachel Harris (Harvard University) Dr Esta van Heerden (iWater)	A fracture fluid sample taken from South Africa resulted in the first almost complete extraction of a *Candidatus bathyarchaeota* genome. This microorganism was found to house genes necessary for carbon fixation via a reductive acetyl Co-A pathway. Other genes located include a periplasmic nitrate reductase (narH), nitrite reductase (nrfHA), and a tungsten-containing formylmethanofuran dehydrogenase (fwdDACB). Methane metabolic gene sequences were located as well.	[56]

Please note that Institution data as shown in each of the individual tables are populated with the most relevant data obtainable for each of the researchers/research institutions involved, rather than the location from whence research had been undertaken at the time of publication.

Supplementary Table 2
Summary of studies conducted on indigenous Biofuel Production and/or Hydrocarbon Degrading strains. Institution data, associated research networks, and reference data

Primary and Secondary Collaborating Institutions	Summary	Associated Publications
Professor IM Tuffin (IMBM) Professor Don Cowan (CMEG)	A pyruvate decarboxylase enzyme from *Gluconobacter oxydans* showing favourable pyruvate affinity with overall high catalytic efficiency (4.75×10^5 M^{-1} s^{-1}) was expressed in the thermophilic strain *Geobacillus thermoglucosidasius*. A fully functional enzyme was observed in the expression host, retaining catalytic activity at 45 °C with final yields that reached as high as 0.35–0.04-g ethanol per gram glucose consumed obtained.	[120]
Professor IM Tuffin (IMBM)	A novel *Trichoderma atroviride* species (strain ES11) was found to grow on low-rank coal, utilizing coal as a sole source of carbon. Biocatalytic studies showed an 82% reduction in coal levels over 21 days	[66]

(*continued on next page*)

Supplementary Table 2 (*continued*)

Primary and Secondary Collaborating Institutions	Summary	Associated Publications
	when cells were cultured in the presence of glucose ($5gL^{-1}$) and low levels of ammonium sulfate ($1gL^{-1}$). Several intracellular depolymerizing enzymes were found to be expressed with 4-hydroxy-phenylethanol, 1.2 benzenediol; and 2-octenoic acid being formed exclusively.	
Ramganesh Selvarajan (University of South Africa)	A *Salinivibrio* species isolated from a Salt Pan water sample in the Free State province (isolate SP9) showed potential for use in the biodegradation of benzanthracene. Metabolic fingerprinting via GC-MS and LC-MS showed the production of 2,3 butanediol, aziridine, ethyl acetate, hexa-hydro-3-(2-methylpropyl) pyrrole [1,2a] pyrazine-1,4-dione and dimethylamine as products. These compounds may be of industrial and pharmaceutical relevance.	[67]

Supplementary Table 3
Summary of studies conducted on indigenous cellulose/ arabinoxylan degrading strains and metagenomic libraries. Institution data, associated research networks, and reference data

Primary and Secondary Collaborating Institutions	Summary	Associated Publications
Dr Tsepo Tsekoa (CSIR) Dr Konanani Rashamuse (DSI)	Metagenomic techniques were used to generate a library of ~70 000 fosmids prepared from DNA extracted from the microbes located in the digestive tract of ruminants. Library screening studies revealed the presence of 2 cellulase genes (cel5A and cel5B) both encoding the production of a 62 kDa thermostable protein that showed an optimum pH of 9.0 and an optimum temperature of 65 °C.	[60]
	A metagenomic expression library created from hindgut symbionts present in *Trinervitermes trinervoides* was screened for the identification of any cellulase/hemicellulose genes. A total of 25 unique cellulase/hemicellulase genes were identified with gene expression studies showing the presence of 4 endo-acting cellulases and 1 exo-acting cellulase in the mix. Another 2 endo-acting xylanases and 1 α-fucosidase enzyme were identified as well.	[71]
Ramganesh Selvarajan (UNISA) Email:	Eighteen unique strains were isolated from a Salt Pan water sample (Free State province). Two of these strains demonstrated cellulase activity when screened using microcrystalline cellulose as substrate. Strain SP7 which showed 100% similarity to a *Halobacillusalkaliphilus* species produced 0.23U/mL cellulase activity upon cultivation while strain SP9 which showed 100% similarity to a *Salinivibrio* sp. Produced 1.95U/mL	[67]
Professor IM Tuffin (IMBM)	Three fosmid libraries prepared from a high-temperature compost sample were evaluated for α-L-arabinofuranosidase activity (AFase). All the AFase enzymes identified showed varied biochemical properties although many showed considerable structural similarities. AFaseD3 showed good thermostability with partial activity retained at 90 °C while AFaseE3 showed the best thermostability with complete activity maintenance at 70 °C.	[72]

Supplementary Table 4
Summary of studies conducted on indigenous Lipase/Esterase-producing strains and metagenomic libraries. Institution data, associated research networks, and reference data

Primary & Secondary Collaborating Institutions	Summary	Associated Publications
Dr Tsepo Tsekoa (CSIR) Dr. Koni Rashamuse (DSI)	Ethyl ferulate hydrolysing (EFH) activity was investigated from *Burkholdaria multivorans*, Preferences for short-chain q-nitrophenyl esters (C2 and C3) were shown. Biocatalytic processes to produce the ferulic from ethyl ferulate were demonstrated as a model substrate.	[73]
	A metagenomic library was constructed and subsequently screened for esterolytic activities. The EstC gene was found represents a member of family VIII esterases with a leader peptide and detectable promiscuous β-lactam hydrolytic activity.	[58]
	Fae6, a feruloyl esterase encoding gene, was derived from a metagenomic library. The high affinities of Fae6 against ethyl ferulate, methyl sinapate, and methyl ferulate suggested that the enzyme could be useful in hydrolysing ferulated polysaccharides in process settings relating to a bio-refinery-type setup.	[74]
	Est22, a novel esterase, derived from an acidic environment was further investigated. Profiling for substrate specificity was conducted using esters (C2–C16) which indicated a preference towards shorter chain *p*-nitrophenyl esters (C2–C5).	[2]
	Termite hindgut symbionts were screened for carboxyl ester hydrolases capable of de-acetylating cephalosporins. Two clones displaying de-acetylating activity belonging to the carbohydrate esterase family 7 (CE7) were identified in this study.	[3]
Professor D Litthauer (UFS) Dr Esta van Heerden (iWater)	Esterolytic activity was screened from a metagenomic library. Two esterolytic clones: a phospholipase patatin protein and an isochorismatase family protein were found. The expressed patatin displayed a preference for the C6 ester and was primarily active at pH 8 and 30 °C.	[57]
Professor IM Tuffin (IMBM) Professor Don Cowan (CMEG)	A metagenomic library from thermophilic compost was constructed in *Escherichia coli.* This library was functionally screened for novel esterases with 25 clones found to demonstrate degradative activity on substrate glyceryl tributyrate. Furthermore, four clones displayed ferulic acid esterase activity. The EstG34 found represents a family of VIII carboxylesterase and β-lactamase fold enzymes, which were able to hydrolyse ferulate and hydroxycinnamic acid esters.	[121]
Ramganesh Selvarajan (University of South Africa)	Culture and molecular approaches were employed to characterise halophilic bacteria from saltpan water samples and profile their potential biotechnological. Quantitative enzyme assays showed moderate extracellular cellulase activity and lipase activity.	[67]

Supplementary Table 5
Summary of studies conducted on indigenous microbial strains demonstrating stereoselective nitrile degrading biocatalytic activity. Institution data, associated research networks, and reference data

Primary and Secondary Collaborating Institutions	Summary	Associated Publications
CSIR Dr. VP Chhiba University of the Witwatersrand Professor Dean Brady	Different β-aminonitriles (3-amino-3-phenylpropanenitrile and derivatives) were produced by the reaction of various benzonitriles with acetonitrile and the reduction of the resulting products. The nitrile hydratase enzyme was indeed enantioselective for these particular compounds, in particular, 3-amino-3-*p*-tolylpropanenitrile and 3-amino-3-(4-methoxyphenyl)propanenitrile and the corresponding amides.	[18]
	Dimethylformamide (DMF) addition during cell cultivation was found to successfully induce nitrilase activity in three different Rhodococcal species namely *R. rhodochrous* ATC BAA 870, *R. rhodococcus* strain A29 as well as *Pimelobacter simplex* strain A99. These indigenous strains together with the solvent-induced nitrilase gene expression regulator create a biological process with a significant reduction in production cost. This has the potential to facilitate large-scale stereoselective nitrile hydrolysis without the need for auxillary/harsh chemicals and high temperature and high-pressure conditions.	[122]
	Biocatalytic kinetic resolution of two beta-hydroxy adrenergic intermediates was attempted using the enantioselective nitrile hydratase and amidase enzymes generated by the organism *R. rhodochrous* ATCC BAA-870. Both amide and acid products formed from 3-hydroxy-4-aryloxy-butanenitriles and 3-hydroxy-3-arylpropanenitriles substrate conversions showed an enantiomeric excess ranging from 0.55 to 99%	[17]
University of the Witwatersrand Professor Dean Brady Tshwane Institute of Technology Dr Renate Roux van der Merwe	Soil samples extracted from various geographical regions within South Africa were subjected to specific enumeration protocols to isolate microorganisms demonstrating nitrile degrading activity. Samples were taken from three different geographical regions, namely from agricultural soil samples, gold mine tailing dams as well as uncultivated soil samples. Elemental limiting growth cultivation techniques were used to isolate microorganisms that grew in the presence of the betahydroxy nitrile substrates.	[19]
CSIR Dr. Tsepo Tsekoa University of Cape Town Professor Trevor Sewell	A thermophilic amidase enzyme produced by *Geobacillus pallidus* RAPc8 was effectively expressed in *E.coli* with high expression levels noted (20U/mg protein). The purified enzyme showed optimum activity at 50°C- 70 °C with D-selectivity against a range of aliphatic amides. Acyl transfer activity was also achieved when using acetamide, isobutyramide, and acrylamide as acyl acceptors.	[82]

References

[1] J. Segobola, E. Adriaenssens, T. Tsekoa, K. Rashamuse, D. Cowan, Exploring viral diversity in a unique South African soil habitat, Sci. Rep. 8 (1) (2018) 111. https://www.nature.com/articles/s41598-017-18461-0.
[2] N. Mokoena, K. Mathiba, T. Tsekoa, P. Steenkamp, K. Rashamuse, Functional characterisation of a metagenome derived family VIII esterase with a deacetylation activity on β-lactam antibiotics, Biochem. Biophys. Res. Commun. 437 (3) (2013) 342–348, https://doi.org/10.1016/j.bbrc.2013.06.076.
[3] N. Mokoena, K. Mathiba, T. Tsekoa, P. Steenkamp, K. Rashamuse, Application of termite hindgut metagenome derived carboxyl ester hydrolases in the modification of cephalosporin substrates, BIochem. Biophy. Rep. 4 (2015) 44–51, https://doi.org/10.1016/j.bbrep.2015.08.016.
[4] Z. Pieterse, T.A. Aveling, A. Jacobs, D.A. Cowan, Diversity and seasonality of fungal communities in soil from the Succulent Karoo biodiversity hotspot, South Africa, J. Arid Environ. 172 (2020), 104020, https://doi.org/10.1016/j.jaridenv.2019.104020.
[5] S.D. Archer, K.C. Lee, T. Caruso, T. Maki, C.K. Lee, S.C. Cary, D.A. Cowan, F.T. Maestre, S.B. Pointing, Airborne microbial transport limitation to isolated Antarctic soil habitats, Nat. Microbiol. 4 (6) (2019) 925–932, https://doi.org/10.1038/s41564-019-0370-4.
[6] P.V. Dunlap, Microbial Diversity, 2001.
[7] P. Lorenz, J. Eck, Metagenomics and industrial applications, Nat. Rev. Microbiol. 3 (6) (2005) 510, https://doi.org/10.1038/nrmicro1161.
[8] S. Roberts, Biocatalysts in synthetic organic chemistry, Tetrahedron 60 (3) (2004) 499–500.
[9] C.J. Clarke, W.-C. Tu, O. Levers, A. Brohl, J.P. Hallett, Green and sustainable solvents in chemical processes, Chem. Rev. 118 (2) (2018) 747–800, https://doi.org/10.1021/acs.chemrev.7b00571.
[10] C. Manyi-Loh, S. Mamphweli, E. Meyer, A. Okoh, Microbial anaerobic digestion: process dynamics and implications from the renewable energy, environmental and agronomy perspectives, Int. J. Environ. Sci. Technol. 16 (2019) 3913–3934, https://doi.org/10.1007/s13762-019-02380-w.
[11] P.T. Anastas, J.C. Warner, Green chemistry, Frontiers 640 (1998) 1998.
[12] A. Valverde, E.D. Cason, A. Gómez-Arias, D. Bozkale, D. Govender, E. Riddell, D. Cowan, Pollution Shapes the Microbial Communities in River Water and Sediments from the Olifants River Catchment, South Africa, Archives of Microbiology, 2020, pp. 1–9, https://doi.org/10.1007/s00203-020-02035-2.
[13] W. Williams, L. Kunorozva, I. Klaiber, M. Henkel, J. Pfannstiel, L.J. Van Zyl, R. Hausmann, A. Burger, M. Trindade, Novel metagenome-derived ornithine lipids identified by functional screening for biosurfactants, Appl. Microbiol. Biotechnol. 103 (11) (2019) 4429–4441, https://doi.org/10.1007/s00253-019-09768-1.
[14] R.M. Matobole, L.J. van Zyl, S. Parker-Nance, M.T. Davies-Coleman, M. Trindade, Antibacterial activities of bacteria isolated from the marine sponges isodictya compressa and Higginsia bidentifera collected from Algoa Bay, South Africa, Mar. Drugs 15 (2) (2017), https://doi.org/10.3390/md15020047.
[15] K. Ndata, W. Nevondo, B. Cekuse, L.J. van Zyl, M. Trindade, Characterization of a highly xylose tolerant β-xylosidase isolated from high temperature horse manure compost, BMC Biotechnol. 21 (1) (2021) 1–10, https://doi.org/10.1186/s12896-021-00722-6.
[16] E.M. Adriaenssens, L.J. Van Zyl, D.A. Cowan, M.I. Trindade, Metaviromics of Namib desert salt pans: a novel lineage of haloarchaeal salterproviruses and a rich source of ssDNA viruses, Viruses 8 (1) (2016) 14, https://doi.org/10.3390/v8010014.
[17] H. Kinfe, V. Chhiba, J. Frederick, M. Bode, K. Mathiba, P. Steenkamp, D. Brady, Enantioselective hydrolysis of β-hydroxy nitriles using the whole cell biocatalyst Rhodococcus rhodochrous ATCC BAA-870, J. Mol. Catal. B Enzym. 59 (4) (2009) 231–236, https://doi.org/10.1016/j.molcatb.2008.06.004.
[18] V. Chhiba, M.L. Bode, K. Mathiba, W. Kwezi, D. Brady, Enantioselective biocatalytic hydrolysis of β-aminonitriles to β-amino-amides using Rhodococcus rhodochrous ATCC BAA-870, J. Mol. Catal. B Enzym. 76 (2012) 68–74, https://doi.org/10.1016/j.molcatb.2011.12.005.
[19] O. Rapheeha, M. Roux-van der Merwe, J. Badenhorst, V. Chhiba, M. Bode, K. Mathiba, D. Brady, Hydrolysis of nitriles by soil bacteria: variation with soil origin, J. Appl. Microbiol. 122 (3) (2017) 686–697, https://doi.org/10.1111/jam.13367.
[20] U. Ramlucken, S.O. Ramchuran, G. Moonsamy, C.J. van Rensburg, M.S. Thantsha, R. Lalloo, Production and stability of a multi-strain Bacillus based probiotic product for commercial use in poultry, Biotechnol. Rep. 29 (2021), e00575, https://doi.org/10.1016/j.btre.2020.e00575.

[21] U. Ramlucken, S.O. Ramchuran, G. Moonsamy, R. Lalloo, M.S. Thantsha, C.J. van Rensburg, A novel Bacillus based multi-strain probiotic improves growth performance and intestinal properties of Clostridium perfringens challenged broilers, Poultry Sci. 99 (1) (2020) 331–341, https://doi.org/10.3382/ps/pez496.

[22] U. Ramlucken, Y. Roets, S.O. Ramchuran, G. Moonsamy, C.J. van Rensburg, M.S. Thantsh, R. Lalloo, Isolation, selection and evaluation of Bacillus spp. as potential multi-mode probiotics for poultry, J. Gen. Appl. Microbiol. 2019 (2) (2020) 11, https://doi.org/10.2323/jgam.2019.11.002.

[23] U. Ramlucken, R. Lalloo, Y. Roets, G. Moonsamy, C.J. van Rensburg, M. Thantsha, Advantages of Bacillus-based probiotics in poultry production, Livest. Sci. 241 (2020), 104215, https://doi.org/10.1016/j.livsci.2020.104215.

[24] R. Lalloo, S. Ramchuran, D. Ramduth, J. Görgens, N. Gardiner, Isolation and selection of Bacillus spp. as potential biological agents for enhancement of water quality in culture of ornamental fish, J. Appl. Microbiol. 103 (5) (2007) 1471–1479, https://doi.org/10.1111/j.1365-2672.2007.03360.x.

[25] R. Lalloo, D. Maharajh, J. Görgens, N. Gardiner, Functionality of a Bacillus cereus biological agent in response to physiological variables encountered in aquaculture, Appl. Microbiol. Biotechnol. 79 (1) (2008) 111–118, https://doi.org/10.1007/s00253-008-1403-8.

[26] R. Lalloo, D. Maharajh, J. Görgens, N. Gardiner, A downstream process for production of a viable and stable Bacillus cereus aquaculture biological agent, Appl. Microbiol. Biotechnol. 86 (2) (2010) 499–508, https://doi.org/10.1007/s00253-009-2294-z.

[27] R. Lalloo, G. Moonsamy, S. Ramchuran, J. Görgens, N. Gardiner, Competitive exclusion as a mode of action of a novel Bacillus cereus aquaculture biological agent, Lett. Appl. Microbiol. 50 (6) (2010) 563–570, https://doi.org/10.1111/j.1472-765X.2010.02829.x.

[28] R. Lalloo, D. Maharajh, J. Görgens, N. Gardiner, J. Görgens, High-density spore production of a B. cereus aquaculture biological agent by nutrient supplementation, Appl. Microbiol. Biotechnol. 83 (1) (2009) 59–66, https://doi.org/10.1007/s00253-008-1845-z.

[29] V.M. Ramdas, P. Mandree, M. Mgangira, S. Mukaratirwa, R. Lalloo, S. Ramchuran, Establishing miniaturised structural testing techniques to enable high-throughput screening of microorganisms and microbial components for unpaved road stabilisation application, J. Adv. Res. 21 (2020) 151–159, https://doi.org/10.1016/j.jare.2019.11.002.

[30] V.M. Ramdas, R. Lalloo, P. Lekha, M. Mgangira, S. Muniyasamy, S. Mukaratirwa, S. Ramchuran, Understanding the mechanism of interaction of candidate soil stabilizing prototypes by using microscopy and spectroscopy techniques, Microsc. Res. Tech. 84 (11) (2021) 2652–2665, https://doi.org/10.1002/jemt.23815.

[31] V.M. Ramdas, P. Mandree, M. Mgangira, S. Mukaratirwa, R. Lalloo, S. Ramchuran, Review of current and future bio-based stabilisation products (enzymatic and polymeric) for road construction materials, Transport. Geotech. 27 (2021), 100458, https://doi.org/10.1016/j.trgeo.2020.100458.

[32] V. Ramdas, R. Lalloo, P. Mandree, M. Mgangira, S. Mukaratirwa, S. Ramchuran, Performance evaluation of bio-based fractions derived from Bacillus spp. for potential in situ soil stabilisation, Appl. Sci. 12 (4) (2022) 1774, https://doi.org/10.3390/app12041774.

[33] P. Mandree, W. Masika, J. Naicker, G. Moonsamy, S. Ramchuran, R. Lalloo, Bioremediation of polycyclic aromatic hydrocarbons from industry contaminated soil using indigenous bacillus spp, Processes 9 (9) (2021) 1606, https://doi.org/10.3390/pr9091606.

[34] W.S. Masika, G. Moonsamy, P. Mandree, S. Ramchuran, R. Lalloo, T. Kudanga, Biodegradation of petroleum hydrocarbon waste using consortia of Bacillus sp, Ann. Finance 25 (1) (2020) 72–79, https://doi.org/10.1080/10889868.2020.1842322.

[35] Y. Roets-Dlamini, R. Lalloo, G. Moonsamy, S. Kumari, M. Nasr, S. Ramchuran, F. Bux, Development of Bacillus spp. consortium for one-step "Aerobic Nitrification-Denitrification" in a fluidized-bed reactor, Bioresour. Technol. Rep. (2022), 100922, https://doi.org/10.1016/j.biteb.2021.100922.

[36] P. Lorenz, K. Liebeton, F. Niehaus, J. Eck, Screening for novel enzymes for biocatalytic processes: accessing the metagenome as a resource of novel functional sequence space, Curr. Opin. Biotechnol. 13 (6) (2002) 572–577, https://doi.org/10.1016/S0958-1669(02)00345-2.

[37] Y. Daya, T. Ranoto, M. Letsoalo, Intra-Africa agricultural trade: a South African perspective, Acta Commer. 6 (1) (2006) 99–113. https://hdl.handle.net/10520/EJC16981.

[38] A.T. Bull, A.C. Ward, M. Goodfellow, Search and discovery strategies for biotechnology: the paradigm shift, Microbiol. Mol. Biol. Rev. 64 (3) (2000) 573–606, https://doi.org/10.1128/MMBR.64.3.573-606.2000.

[39] S. Mtimka, P. Pillay, K. Rashamuse, S. Gildenhuys, T.L. Tsekoa, Functional screening of a soil metagenome for DNA endonucleases by acquired resistance to bacteriophage infection, Mol. Biol. Rep. 47 (1) (2020) 353–361, https://doi.org/10.1007/s11033-019-05137-3.

[40] M. Medjemadj, J.-J. Escuder-Rodríguez, A. Boudemagh, M.-I. González-Siso, Actinobacteria isolated from Algerian hot spring waters: a potential source of important enzymes, Ecol. Environ. Conserv. 26 (3) (2020) 1145–1157.

[41] E. Mathur, C. Constanza, L. Christoffersen, C. Erikson, M. Sullivan, M. Bene, J. Short, An overview of bioprospecting and the Diversa model, IP Strat. Today 4 (2004).

[42] J.-K. Preem, J. Truu, M. Truu, Ü. Mander, K. Oopkaup, K. Lõhmus, H.-S. Helmisaari, V. Uri, M. Zobel, Bacterial community structure and its relationship to soil physico-chemical characteristics in alder stands with different management histories, Ecol. Eng. 49 (2012) 10–17, https://doi.org/10.1016/j.ecoleng.2012.08.034.

[43] L.-L. Li, S.R. McCorkle, S. Monchy, S. Taghavi, D. van der Lelie, Bioprospecting metagenomes: glycosyl hydrolases for converting biomass, Biotechnol. Biofuels 2 (1) (2009) 10, https://doi.org/10.1186/1754-6834-2-10.

[44] E.P. Culligan, R.D. Sleator, J.R. Marchesi, C. Hill, Metagenomics and novel gene discovery: promise and potential for novel therapeutics, Virulence 5 (3) (2014) 399–412, https://doi.org/10.4161/viru.27208.

[45] X. Fan, X. Liu, R. Huang, Y. Liu, Identification and characterization of a novel thermostable pyrethroid-hydrolyzing enzyme isolated through metagenomic approach, Microb. Cell Factories 11 (1) (2012) 33. http://www.microbialcellfactories.com/content/11/1/33.

[46] T. Prakash, T.D. Taylor, Functional assignment of metagenomic data: challenges and applications, Briefings Bioinf. 13 (6) (2012) 711–727, https://doi.org/10.1093/bib/bbs033.

[47] H. Jung, T. Ventura, J.S. Chung, W.-J. Kim, B.-H. Nam, H.J. Kong, Y.-O. Kim, M.-S. Jeon, S.-i. Eyun, Twelve quick steps for genome assembly and annotation in the classroom, PLoS Comput. Biol. 16 (11) (2020), e1008325, https://doi.org/10.1371/journal.pcbi.1008325.

[48] J.T. Staley, A. Konopka, Measurement of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats, Annu. Rev. Microbiol. 39 (1) (1985) 321–346, https://doi.org/10.1146/annurev.mi.39.100185.001541.

[49] J. Handelsman, Metagenomics: application of genomics to uncultured microorganisms, Microbiol. Mol. Biol. Rev. 68 (4) (2004) 669–685, https://doi.org/10.1128/MMBR.68.4.669-685.2004.

[50] B.K. Singh, Exploring microbial diversity for biotechnology: the way forward, Trends Biotechnol. 28 (3) (2010) 111–116, https://doi.org/10.1016/j.tibtech.2009.11.006.

[51] E.M. Adriaenssens, L. Van Zyl, P. De Maayer, E. Rubagotti, E. Rybicki, M. Tuffin, D.A. Cowan, Metagenomic analysis of the viral community in Namib Desert hypoliths, Environ. Microbiol. 17 (2) (2015) 480–495, https://doi.org/10.1111/1462-2920.12528.

[52] U. Hesse, P. Van Heusden, B.M. Kirby, I. Olonade, L.J. Van Zyl, M. Trindade, Virome assembly and annotation: a surprise in the Namib Desert, Front. Microbiol. 8 (2017) 13, https://doi.org/10.3389/fmicb.2017.00013.

[53] V. Scola, J.-B. Ramond, A. Frossard, O. Zablocki, E.M. Adriaenssens, R.M. Johnson, M. Seely, D.A. Cowan, Namib desert soil microbial community diversity, assembly, and function along a natural xeric gradient, Microb. Ecol. 75 (1) (2018) 193–203, https://doi.org/10.1007/s00248-017-1009-8.

[54] L. Martínez-Alvarez, J. Ramond, S. Vikram, C. León-Sobrino, G. Maggs-Kölling, D. Cowan, With a Pinch of Salt: Metagenomic Insights into Namib Desert Salt pan Microbial Mats and Halites Reveal Functionally Adapted and Competitive Communities, 2022, https://doi.org/10.1101/2022.02.18.481119.

[55] C. Magnabosco, M. Tekere, M.C. Lau, B. Linage, O. Kuloyo, M. Erasmus, E. Cason, E. van Heerden, G. Borgonie, T.L. Kieft, Comparisons of the composition and biogeographic distribution of the bacterial communities occupying South African thermal springs with those inhabiting deep subsurface fracture water, Front. Microbiol. 5 (2014) 679. https://www.frontiersin.org/articles/10.3389/fmicb.2014.00679/full.

[56] R.L. Harris, M.C. Lau, A. Cadar, D.H. Bartlett, E. Cason, E. Van Heerden, T.C. Onstott, Draft genome sequence of "Candidatus Bathyarchaeota" archaeon BE326-BA-RLH, an uncultured denitrifier and putative anaerobic methanotroph from South Africa's deep continental biosphere, Microbiol. Res. Announc. 7 (20) (2018), e01295, https://doi.org/10.1128/MRA.01295-18.

[57] N.S. Abbai, E. van Heerden, L.A. Piater, D. Litthauer, A deep gold mine metagenome as a source of novel esterases, Afr. J. Biotechnol. 10 (32) (2011) 6090–6100, https://doi.org/10.5897/AJB10.1211.

[58] K. Rashamuse, V. Magomani, T. Ronneburg, D. Brady, A novel family VIII carboxylesterase derived from a leachate metagenome library exhibits promiscuous β-lactamase activity on nitrocefin, Appl. Microbiol. Biotechnol. 83 (3) (2009) 491–500, https://doi.org/10.1007/s00253-009-1895-x.

[59] T.W. Sanyika, K.J. Rashamuse, F. Hennessy, D. Brady, Luminal hindgut bacterial diversities of the grass and sugarcane feeding termite Trinervitermes trinervoides, Afr. J. Microbiol. Res. 6 (11) (2012) 2639–2648, https://doi.org/10.5897/AJMR11.975.

[60] K. Rashamuse, D.F. Visser, F. Hennessy, J. Kemp, M. Roux-Van Der Merwe, J. Badenhorst, T. Ronneburg, R. Francis-Pope, D. Brady, Characterisation of two bifunctional cellulase–xylanase enzymes isolated from a bovine rumen metagenome library, Curr. Microbiol. 66 (2) (2013) 145–151, https://doi.org/10.1007/s00284-012-0251-z.

[61] L.J. van Zyl, Y. Abrahams, E.A. Stander, B. Kirby-McCollough, R. Jourdain, C. Clavaud, L. Breton, M. Trindade, Novel phages of healthy skin metaviromes from South Africa, Sci. Rep. 8 (1) (2018) 1–13, https://doi.org/10.1038/s41598-018-30705-1.

[62] L.J. Van Zyl, W.-D. Schubert, M.I. Tuffin, D.A. Cowan, Structure and functional characterization of pyruvate decarboxylase from Gluconacetobacter diazotrophicus, BMC Struct. Biol. 14 (1) (2014) 1–13. https://bmcstructbiol.biomedcentral.com/articles/10.1186/s12900-014-0021-1.

[63] R.U. Meckenstock, E. Annweiler, W. Michaelis, H.H. Richnow, B. Schink, Anaerobic naphthalene degradation by a sulfate-reducing enrichment culture, Appl. Environ. Microbiol. 66 (7) (2000) 2743–2747, https://doi.org/10.1128/AEM.66.7.2743-2747.2000.

[64] R. Rabus, M. Boll, J. Heider, R.U. Meckenstock, W. Buckel, O. Einsle, U. Ermler, B.T. Golding, R.P. Gunsalus, P.M. Kroneck, Anaerobic microbial degradation of hydrocarbons: from enzymatic reactions to the environment, Microb. Physiol. 26 (1–3) (2016) 5–28, https://doi.org/10.1159/000443997.

[65] R.U. Meckenstock, M. Boll, H. Mouttaki, J.S. Koelschbach, P.C. Tarouco, P. Weyrauch, X. Dong, A.M. Himmelberg, Anaerobic degradation of benzene and polycyclic aromatic hydrocarbons, Microb. Physiol. 26 (1–3) (2016) 92–118, https://doi.org/10.1159/000441358.

[66] M.E. Silva-Stenico, C.J. Vengadajellum, H.A. Janjua, S.T. Harrison, S.G. Burton, D.A. Cowan, Degradation of low rank coal by Trichoderma atroviride ES11, J. Ind. Microbiol. Biotechnol. 34 (9) (2007) 625–631, https://doi.org/10.1007/s10295-007-0223-7.

[67] R. Selvarajan, T. Sibanda, M. Tekere, H. Nyoni, S. Meddows-Taylor, Diversity analysis and bioresource characterization of halophilic bacteria isolated from a South African saltpan, Molecules 22 (4) (2017) 657, https://doi.org/10.3390/molecules22040657.

[68] M. Cabrera, J.M. Blamey, Biotechnological applications of archaeal enzymes from extreme environments, Biol. Res. 51 (2018) 37–51, https://doi.org/10.1186/s40659-018-0186-3.

[69] R.C. Kuhad, R. Gupta, A. Singh, Microbial cellulases and their industrial applications, Enzym. Res. 2011 (2011), https://doi.org/10.4061/2011/280696.

[70] A.B. Gunjal, N.N. Patil, S.S. Shinde, Enzymes in Degradation of the Lignocellulosic Wastes, Springer, 2020.

[71] K. Rashamuse, W. Sanyika Tendai, K. Mathiba, T. Ngcobo, S. Mtimka, D. Brady, Metagenomic mining of glycoside hydrolases from the hindgut bacterial symbionts of a termite (Trinervitermes trinervoides) and the characterization of a multimodular β-1, 4-xylanase (GH11), Biotechnol. Appl. Biochem. 64 (2) (2017) 174–186, https://doi.org/10.1002/bab.1480.

[72] B. Fortune, S. Mhlongo, L.J. van Zyl, R. Huddy, M. Smart, M. Trindade, Characterisation of three novel α-L-arabinofuranosidases from a compost metagenome, BMC Biotechnol. 19 (1) (2019) 1–13. https://bmcbiotechnol.biomedcentral.com/articles/10.1186/s12896-019-0510-1.

[73] K. Rashamuse, S. Burton, D. Cowan, A novel recombinant ethyl ferulate esterase from Burkholderia multivorans, J. Appl. Microbiol. 103 (5) (2007) 1610–1620, https://doi.org/10.1111/j.1365-2672.2007.03394.x.

[74] K. Rashamuse, W. Sanyika, T. Ronneburg, D. Brady, A feruloyl esterase derived from a leachate metagenome library, BMB Rep. 45 (1) (2012) 14–19, https://doi.org/10.5483/BMBRep.2012.45.1.14.

[75] C. Brenner, Hint, Fhit, and GalT: function, structure, evolution, and mechanism of three branches of the histidine triad superfamily of nucleotide hydrolases and transferases, Biochemistry 41 (29) (2002) 9003–9014, https://doi.org/10.1021/bi025942q.

[76] H.C. Pace, C. Brenner, The nitrilase superfamily: classification, structure and function, Genome Biol. 2 (1) (2001) 1–9, https://doi.org/10.1186/gb-2001-2-1-reviews0001.

[77] M. Nishiyama, S. Horinouchi, M. Kobayashi, T. Nagasawa, H. Yamada, T. Beppu, Cloning and characterization of genes responsible for metabolism of nitrile compounds from Pseudomonas chlororaphis B23, J. Bacteriol. 173 (8) (1991) 2465–2472, https://doi.org/10.1128/jb.173.8.2465-2472.1991.

[78] M.S. Payne, S. Wu, R.D. Fallon, G. Tudor, B. Stieglitz, I.M. Turner, M.J. Nelson, A stereoselective cobalt-containing nitrile hydratase, Biochemistry 36 (18) (1997) 5447–5454, https://doi.org/10.1021/bi962794t.

[79] T. Yamaki, T. Oikawa, K. Ito, T. Nakamura, Cloning and sequencing of a nitrile hydratase gene from Pseudonocardia thermophila JCM3095, J. Ferment. Bioeng. 83 (5) (1997) 474–477, https://doi.org/10.1016/S0922-338X(97)83004-8.

[80] O. Ikehata, M. Nishiyama, S. Horinouchi, T. Beppu, Primary structure of nitrile hydratase deduced from the nucleotide sequence of a Rhodococcus species and its expression in Escherichia coli, Eur. J. Biochem. 181 (3) (1989) 563–570, https://doi.org/10.1111/j.1432-1033.1989.tb14761.x.

[81] T. Nagasawa, H. Shimizu, H. Yamada, The superiority of the third-generation catalyst, Rhodococcus rhodochrous J1 nitrile hydratase, for industrial production of acrylamide, Appl. Microbiol. Biotechnol. 40 (2) (1993) 189–195, https://doi.org/10.1007/BF00170364.

[82] H. Makhongela, A. Glowacka, V. Agarkar, B. Sewell, B. Weber, R. Cameron, D. Cowan, S. Burton, A novel thermostable nitrilase superfamily amidase from Geobacillus pallidus showing acyl transfer activity, Appl. Microbiol. Biotechnol. 75 (4) (2007) 801–811, https://doi.org/10.1007/s00253-007-0883-2.

[83] R. Jaenicke, Enzymes under extremes of physical conditions, Annu. Rev. Biophys. Bioeng. 10 (1) (1981) 1–67, https://doi.org/10.1146/annurev.bb.10.060181.000245.

[84] M. Ferrer, O. Golyshina, A. Beloqui, P.N. Golyshin, Mining enzymes from extreme environments, Curr. Opin. Microbiol. 10 (3) (2007) 207–214, https://doi.org/10.1016/j.mib.2007.05.004.

[85] A. Valverde, M. Tuffin, D.A. Cowan, Biogeography of bacterial communities in hot springs: a focus on the actinobacteria, Extremophiles 16 (4) (2012) 669–679. https://link.springer.com/article/10.1007/s00792-012-0465-9.

[86] F. Stomeo, A. Valverde, S.B. Pointing, C.P. Mckay, K.A. Warren-rhodes, M.I. Tuffin, M. Seely, D.A. Cowan, Hypolithic and soil microbial community assembly along an aridity gradient in the Namib Desert, Extremophiles 17 (2) (2013) 329, https://doi.org/10.1007/s00792-013-0519-7.

[87] J.-B. Ramond, P.J. Welz, M. Le Roes-Hill, M.I. Tuffin, S.G. Burton, D.A. Cowan, Selection of Clostridium spp. in biological sand filters neutralizing synthetic acid mine drainage, FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol. 87 (3) (2014) 678–690, https://doi.org/10.1111/1574-6941.12255.

[88] J.B. Ramond, J.D. Lako, W.H. Stafford, M.I. Tuffin, D.A. Cowan, Evidence of novel plant-species specific ammonia oxidizing bacterial clades in acidic South African fynbos soils, J. Basic Microbiol. 55 (8) (2015) 1040–1047, https://doi.org/10.1002/jobm.201400933.

[89] T.P. Makhalanyane, A. Valverde, D.C. Lacap, S.B. Pointing, M.I. Tuffin, D.A. Cowan, Evidence of species recruitment and development of hot desert hypolithic communities, Environ. Micorbiol. Rep. 5 (2) (2013) 219–224, https://doi.org/10.1111/1758-2229.12003.

[90] O. Zablocki, L. van Zyl, M. Trindade, Biogeography and taxonomic overview of terrestrial hot spring thermophilic phages, Extremophiles 22 (6) (2018) 827–837, https://doi.org/10.1007/s00792-018-1052-5.

[91] G.B. Slobodkina, O.L. Kovaleva, M.L. Miroshnichenko, A.I. Slobodkin, T.V. Kolganova, A.A. Novikov, E. van Heerden, E.A. Bonch-Osmolovskaya, Thermogutta terrifontis gen. nov., sp. nov. and Thermogutta hypogea sp. nov., thermophilic anaerobic representatives of the phylum Planctomycetes, Int. J. Syst. Evol. Microbiol. 65 (Pt_3) (2015) 760–765, https://doi.org/10.1099/ijs.0.000009.

[92] O.A. Podosokorskaya, A.Y. Merkel, S.N. Gavrilov, I. Fedoseev, E. van Heerden, E.D. Cason, A.A. Novikov, T.V. Kolganova, A.A. Korzhenkov, E.A. Bonch-Osmolovskaya, Tepidibacillus infernus sp. nov., a moderately thermophilic, selenate-and arsenate-respiring hydrolytic bacterium isolated from a gold mine, and emended description of the genus Tepidibacillus, Int. J. Syst. Evol. Microbiol. 66 (8) (2016) 3189–3194, https://doi.org/10.1099/ijsem.0.001166.

[93] P.J. Welz, J.-B. Ramond, D.A. Cowan, S.G. Burton, M. Le Roes-Hill, Minor differences in sand physicochemistry lead to major differences in bacterial community structure and function after exposure to synthetic acid mine drainage, Biotechnol. Bioproc. Eng. 19 (2) (2014) 211–220. https://link.springer.com/article/10.1007/s12257-013-0454-6.

[94] M. Soliman, A. Eldyasti, Ammonia-Oxidizing Bacteria (AOB): opportunities and applications—a review, Rev. Environ. Sci. Biotechnol. 17 (2) (2018) 285–321. https://link.springer.com/article/10.1007/s11157-018-9463-4.

[95] A. Mishra, A. Malik, Recent advances in microbial metal bioaccumulation, Crit. Rev. Environ. Sci. Technol. 43 (11) (2013) 1162–1222, https://doi.org/10.1080/10934529.2011.627044.

[96] S. Gunatilake, Methods of removing heavy metals from industrial wastewater, Methods 1 (1) (2015) 14.
[97] A. Demir, M. Arisoy, Biological and chemical removal of Cr (VI) from waste water: cost and benefit analysis, J. Hazard Mater. 147 (1–2) (2007) 275–280, https://doi.org/10.1016/j.jhazmat.2006.12.076.
[98] F. Fu, Q. Wang, Removal of heavy metal ions from wastewaters: a review, J. Environ. Manag. 92 (3) (2011) 407–418, https://doi.org/10.1016/j.jenvman.2010.11.011.
[99] B.K. Chipasa, Accumulation and Fate of Selected Heavy Metals in Biological Wastewater Treatment System, 2005, https://doi.org/10.1016/S0956-053X(02)00065-X.
[100] H. Qin, T. Hu, Y. Zhai, N. Lu, J. Aliyeva, The improved methods of heavy metals removal by biosorbents: a review, Environ. Pollut. 258 (2020), 113777, https://doi.org/10.1016/j.envpol.2019.113777.
[101] S. Staniland, M. Coppock, M. Tuffin, L. van Zyl, A.N. Roychoudhury, D. Cowan, Cobalt uptake and resistance to trace metals in Comamonas testosteroni isolated from a heavy-metal contaminated site in the Zambian copperbelt, Geomicrobiol. J. 27 (8) (2010) 656–668, https://doi.org/10.1080/01490450903527994.
[102] M. Maleke, A. Valverde, J.-G. Vermeulen, E. Cason, A. Gomez-Arias, K. Moloantoa, L. Coetsee-Hugo, H. Swart, E. Van Heerden, J. Castillo, Biomineralization and bioaccumulation of europium by a thermophilic metal resistant bacterium, Front. Microbiol. (2019) 81, https://doi.org/10.3389/fmicb.2019.00081.
[103] E.D. Cason, L.A. Piater, E. van Heerden, Reduction of U (VI) by the deep subsurface bacterium, Thermus scotoductus SA-01, and the involvement of the ABC transporter protein, Chemosphere 86 (6) (2012) 572–577, https://doi.org/10.1016/j.chemosphere.2011.10.006.
[104] M.O. Agunbiade, C. Pohl, E.V. Heerden, O. Oyekola, A. Ashafa, Evaluation of fresh water actinomycete bioflocculant and its biotechnological applications in wastewaters treatment and removal of heavy metals, Int. J. Environ. Res. Publ. Health 16 (18) (2019) 3337, https://doi.org/10.3390/ijerph16183337.
[105] M. Erasmus, E.D. Cason, J. van Marwijk, E. Botes, M. Gericke, E. van Heerden, Gold nanoparticle synthesis using the thermophilic bacterium Thermus scotoductus SA-01 and the purification and characterization of its unusual gold reducing protein, Gold Bull. 47 (4) (2014) 245–253. https://link.springer.com/article/10.1007/s13404-014-0147-8.
[106] T.A. Brown, Mutation, repair and recombination, in: Genomes, second ed., Wiley-Liss, 2002.
[107] P.J. Rothwell, G. Waksman, Structure and mechanism of DNA polymerases, Adv. Protein Chem. 71 (2005) 401–440, https://doi.org/10.1016/S0065-3233(04)71011-6.
[108] G. Pergolizzi, G.K. Wagner, R.P. Bowater, Biochemical and structural characterization of DNA ligases from bacteria and archaea, Biosci. Rep. 36 (5) (2016), https://doi.org/10.1042/BSR20160003.
[109] F. Cortés, N. Pastor, S. Mateos, I. Domınguez, Roles of DNA topoisomerases in chromosome segregation and mitosis, Mutat. Res., Rev. Mutat. Res. 543 (1) (2003) 59–66, https://doi.org/10.1016/S1383-5742(02)00070-4.
[110] W. Yang, Nucleases: diversity of structure, function and mechanism, Q. Rev. Biophys. 44 (1) (2011) 1–93, https://doi.org/10.1017/s0033583510000181.
[111] S.S. Dewan, Life Science Tools and Reagents: Global Markets, 2021. Available from: https://www.bccresearch.com/market-research/biotechnology/life-science-tools-reagents-markets-report.html.
[112] M.P.J. Segobola, Identification and Characterisation of Nucleic Acid Manipulating Enzymes from Metaviromic DNA, University of Pretoria, 2019.
[113] M.I. Mokoena, Mining of Genes Encoding for DNA-Manipulating Enzymes from Hot Springs Using Metagenomic Techniques, Vaal University of Technology, 2019.
[114] M.B. Holowko, E.K. Frow, J.C. Reid, M. Rourke, C.E. Vickers, Building a biofoundry, Synth. Biol. 6 (1) (2021) ysaa026, https://doi.org/10.1093/synbio/ysaa026.
[115] S. Herrera, Industrial biotechnology—a chance at redemption, Nat. Biotechnol. 22 (6) (2004) 671.
[116] K. Rashamuse, T. Ronneburg, W. Sanyika, K. Mathiba, E. Mmutlane, D. Brady, Metagenomic mining of feruloyl esterases from termite enteric flora, Appl. Microbiol. Biotechnol. 98 (2) (2014) 727–737, https://doi.org/10.1007/s00253-013-4909-7.
[117] I. Olonade, L.J. van Zyl, M. Trindade, Genomic characterization of a prophage, Smhb1, that infects Salinivibrio kushneri BNH isolated from a Namib Desert saline spring, Microorganisms 9 (10) (2021) 2043, https://doi.org/10.3390/microorganisms9102043.
[118] O. Zablocki, L.J. Van Zyl, B. Kirby, M. Trindade, Diversity of dsDNA viruses in a South African hot spring assessed by metagenomics and microscopy, Viruses 9 (11) (2017) 348, https://doi.org/10.3390/v9110348.
[119] O. Zablocki, E.M. Adriaenssens, A. Frossard, M. Seely, J.-B. Ramond, D. Cowan, Metaviromes of extracellular soil viruses along a Namib Desert aridity gradient, Genome Announc. 5 (2) (2017), e01470, https://doi.org/10.1128/genomeA.01470-16.
[120] L.J. Van Zyl, M. Taylor, K. Eley, M. Tuffin, D.A. Cowan, Engineering pyruvate decarboxylase-mediated ethanol production in the thermophilic host Geobacillus thermoglucosidasius, Appl. Microbiol. Biotechnol. 98 (3) (2014) 1247–1259, https://doi.org/10.1007/s00253-013-5380-1.
[121] C.W. Ohlhoff, B.M. Kirby, L. Van Zyl, D.L. Mutepfa, A. Casanueva, R.J. Huddy, R. Bauer, D.A. Cowan, M. Tuffin, An unusual feruloyl esterase belonging to family VIII esterases and displaying a broad substrate range, J. Mol. Catal. B Enzym. 118 (2015) 79–88, https://doi.org/10.1016/j.molcatb.2015.04.010.
[122] V. Chhiba-Govindjee, K. Mathiba, C.W. Van der Westhuyzen, P. Steenkamp, J. Rashamuse, S. Stoychev, M. Bode, D. Brady, Dimethylformamide is a novel nitrilase inducer in Rhodococcus rhodochrous, Appl. Microbiol. Biotechnol. 102 (23) (2018) 10055–10065, https://doi.org/10.1007/s00253-018-9367-9.