

Review

Metagenomic Approaches as a Tool to Unravel Promising Biocatalysts from Natural Resources: Soil and Water

Joana Sousa ^{1,2}, Sara C. Silvério ^{1,2,*}, Angela M. A. Costa ^{1,2} and Ligia R. Rodrigues ^{1,2}

¹ CEB—Centre of Biological Engineering, Universidade do Minho, 4710-057 Braga, Portugal; joanarfs@hotmail.com (J.S.); angelacostinha@gmail.com (A.M.A.C.); lrmr@deb.uminho.pt (L.R.R.)

² LABBELS—Associate Laboratory, 4710-057 Braga, Portugal

* Correspondence: sarasilverio@deb.uminho.pt

Abstract: Natural resources are considered a promising source of microorganisms responsible for producing biocatalysts with great relevance in several industrial areas. However, a significant fraction of the environmental microorganisms remains unknown or unexploited due to the limitations associated with their cultivation in the laboratory through classical techniques. Metagenomics has emerged as an innovative and strategic approach to explore these unculturable microorganisms through the analysis of DNA extracted from environmental samples. In this review, a detailed discussion is presented on the application of metagenomics to unravel the biotechnological potential of natural resources for the discovery of promising biocatalysts. An extensive bibliographic survey was carried out between 2010 and 2021, covering diverse metagenomic studies using soil and/or water samples from different types and locations. The review comprises, for the first time, an overview of the worldwide metagenomic studies performed in soil and water and provides a complete and global vision of the enzyme diversity associated with each specific environment.

Keywords: soil; water; metagenomics; enzymes; biodiversity



Citation: Sousa, J.; Silvério, S.C.; Costa, A.M.A.; Rodrigues, L.R. Metagenomic Approaches as a Tool to Unravel Promising Biocatalysts from Natural Resources: Soil and Water. *Catalysts* **2022**, *12*, 385. <https://doi.org/10.3390/catal12040385>

Academic Editors: Chia-Hung Kuo, Chun-Yung Huang, Chwen-Jen Shieh and Cheng-Di Dong

Received: 17 February 2022

Accepted: 27 March 2022

Published: 30 March 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The natural resources available on Earth have played an important role in the history of human civilization since they have provided the necessary materials and energy for the preservation and proliferation of life. Besides the basic sustenance, proper use of these resources can also contribute to the improvement of our comfort, protection and well-being [1]. Over the centuries, we have witnessed the continuous exploitation of some finite natural resources, which resulted in the degradation of important ecosystems, subsequently creating severe environmental, economic and technological issues [2,3]. This overconsumption of materials and energy will eventually lead to inevitable resource depletion. Consequently, there is a need to moderate the demand for finite natural resources and simultaneously search for efficient and sustainable ways to extract and convert energy/materials from renewable resources [4]. In this context, biocatalysts can play a crucial role. Due to their high specificity and selectivity, the biocatalysts can generally ensure the effective conversion of substrates, minimizing the formation of undesirable side-products and reducing the energetic costs associated with the process. Hence, the use of suitable and robust biocatalysts can greatly contribute to the implementation of greener and sustainable bioprocesses that efficiently compete with the classical chemical routes. Currently, the use of biocatalysts for the valorization of alternative non-finite resources, under the concept of bioeconomy and the EU Green Deal, has gained increased attention.

Unexplored or slightly explored environments, such as soil and water, are interesting sources of novel and promising biocatalysts. Despite the clear differences at the physicochemical level, soil and water are both regarded as natural bio-reservoirs with great microbial diversity. For this reason, these environments have been the focus of several microbial studies in the last few decades. Microorganisms are considered important suppliers

of various bioproducts with applications in several industrial areas, such as enzymes. In fact, in the last decade, we have seen a significant increase in the demand for enzymes [5], which is easily explained by their great biotechnological potential. However, the presence of a significant number of unculturable microorganisms both in the soil and water can limit or make unfeasible some microbial studies to find novel biocatalysts. In this context, metagenomics can play a crucial role.

Metagenomics has emerged as a culture-independent technique that allows exploring the genetic material of whole microbial communities present in a given environment [6]. This technique has been successfully used to identify novel enzymes with promising catalytic activities and some of them have been patented and already translated to the market [7]. Two different metagenomic approaches have been described, namely, sequence-based or function-based metagenomics. In both cases, an initial step of DNA extraction from an environmental sample is needed [8]. The sequence-based studies allow the identification of candidate genes, while the function-based screenings include the detection and isolation of clones from metagenomic libraries with a positive response to the desired phenotype [9]. The construction of a metagenomic library requires the selection of the most suitable expression vector, in which the environmental DNA fragment will be inserted. In addition to other aspects, such as the quality and size of the environmental DNA, this selection depends on the purpose of the functional screening. Plasmids can be used when DNA fragments are small (≤ 15 kb insert size) and contain only individual genes. On the other hand, some expression vectors, such as fosmids and cosmids (< 40 kb insert size), or bacterial artificial chromosomes (BACs) and yeast artificial chromosomes (YACs) (> 40 kb insert size), allow the recovery of large biosynthetic gene clusters that encode the production of one or more specialized metabolites. Besides the expression vector, expression systems should be selected in such a way that gene expression and target gene detection are maximized. *Escherichia coli* has been widely used due to the extensive genetic knowledge on this microorganism that makes it suitable for effective and profitable cloning and protein expression [10,11]. However, the demand for robust biocatalysts requires functional screenings at temperatures other than the growth temperatures of mesophilic hosts. Therefore, other expression hosts, such as *Thermus thermophilus*, have already been shown to be good candidates as expression hosts in the functional metagenome analysis [12].

This review discusses the most significant works reported in the last decade about metagenomic studies performed in soil and water environments for the discovery of novel and interesting enzymes. The bibliographic survey was carried out in the international database Scopus between 2010 and 2021 using several search criteria as illustrated in Figure 1. Briefly, in the first criteria the keywords used were: “metagenomic” and “enzyme” and “soil” or “water”. After that, for each natural resource, the search was refined according to the type and origin of the samples. Finally, the different groups of enzymes were considered in the search.

INTERNATIONAL DATABASE SCOPUS	
YEARS: 2010–2021	LANGUAGE: ENGLISH
TYPE: ARTICLE	
SOIL	WATER
KEYWORDS:	
1 st Search Criteria	1 st Search Criteria
"Metagenomic" and "Enzyme" and "Soil"	"Metagenomic" and "Enzyme" and "Water"
2 nd Search Criteria	2 nd Search Criteria
("Metagenomic" and "Enzyme" and "Soil") and "Forest" or "Mud" or "Desert" or "Glacial" or "Mountain" or "Land" or "Compost" or "Contaminated" or "Sludge" or "Sediment"	("Metagenomic" and "Enzyme" and "Water") and "Marine" or "Aquatic" or "Contaminated"
3 rd Search Criteria	3 rd Search Criteria
("Metagenomic" and "Enzyme" and "Soil") and "Glycosyl hydrolase" or "Cellulase" or "Xylanase" or "Amylase" or "Chitinase" or "Oxidoreductase" or "Laccase" or "Lipolytic" or "Lipase" or "Esterase" or "Phosphatase" or "Phytase" or "Protease"	("Metagenomic" and "Enzyme" and "Water") and "Glycosyl hydrolase" or "Cellulase" or "Xylanase" or "Amylase" or "Chitinase" or "Oxidoreductase" or "Laccase" or "Lipolytic" or "Lipase" or "Esterase" or "Phosphatase" or "Phytase" or "Protease"

Figure 1. Search criteria defined for the bibliographic survey carried out in the international database Scopus. The first research criteria consisted of a generic search for metagenomic studies involving enzymes; the second criteria restricted the search to different types of samples and their origins; and the last criteria focused on the different groups of enzymes. In each case, the established keywords were searched in the title, abstract or keywords of the article.

Based on the results obtained in the bibliographic survey, the studies were divided into three categories according to the type of sample (e.g., raw resources, human manipulated resources and unspecified resources) as illustrated in Figure 2. Within the category of raw resources, differences were established according to the temperature of each specific environment. In the case of human manipulated resources, three conditions were considered for soil, namely, samples from polluted environments, composting processes and agricultural lands and grassland. For the water samples resulting from human manipulation two conditions were defined, namely, contaminated groundwater/freshwater and coastal water. In the category of unspecified resources, both for soil and water, all studies that did not present enough information to classify them according to the categories mentioned above were included. Overall, for each study, the environmental conditions (temperature and pH), the type of metagenomic approach and its main characteristics, as well as the catalytic activity, was revised. Furthermore, the number of citations found in the international database Scopus was also presented as an indicator of the impact of the study. All this information was summarized in the tables presented in each specific subsection.

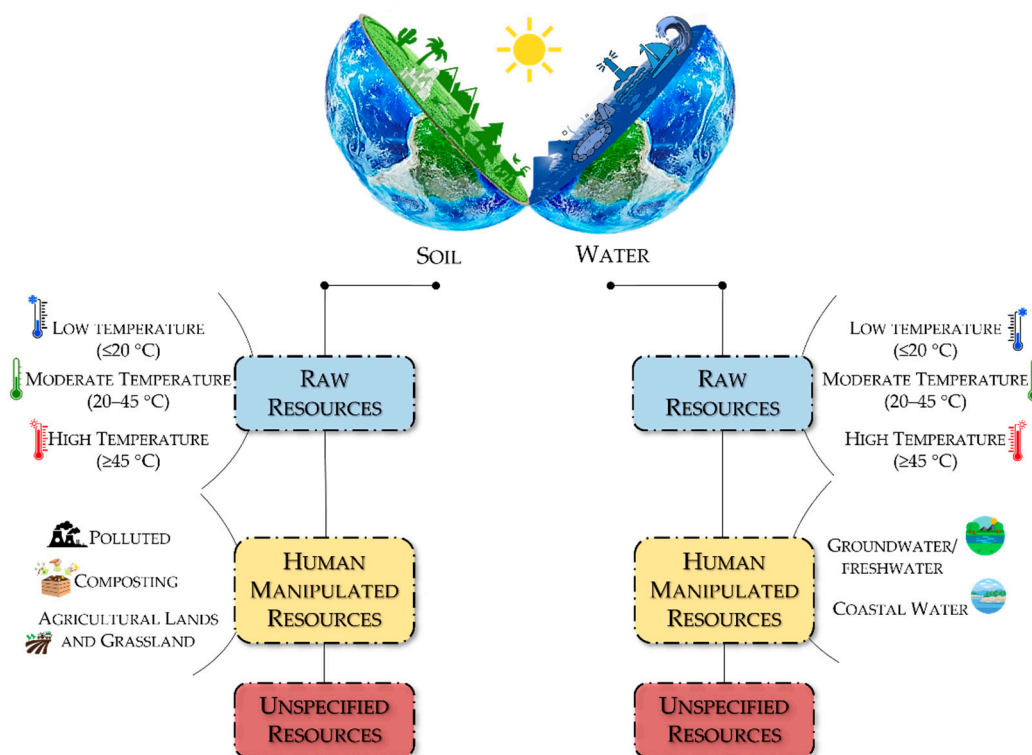


Figure 2. Classification of the different soil and water sources used in metagenomic studies conducted to identify promising biocatalysts.

2. Soil

Soil is more than just land; it represents one of the most important natural resources available on Earth. It is considered the basis of agriculture, an important water filter, a natural reserve of carbon and water and also the regular habitat for several living organisms. Soils originate from rocks through complex chemical, mechanical and biological processes which result in the formation of small particles and grains [13]. In terms of composition, soils are generally constituted by solid, liquid and gas fractions in a 2:1:1 ratio (volume basis). The solid fraction is mainly composed of inorganic materials but also contains a small portion of organic matter (around 1–5%) [14]. The liquid phase is generally an aqueous solution of electrolytes (e.g., micronutrients and macronutrients). On the other hand, the main gases present in soil are water vapor, CO₂, O₂ and N₂ [13]. Depending on its origin and composition, different types of soil can be defined. Microbial diversity and functionality are strongly dependent on the specific characteristics of each type of soil. The classification established for the different soil samples considered in this review is presented in Figure 3a. A total of 173 metagenomic studies were performed, considering soil samples aiming to explore promising enzymes.

As shown in Figure 3b, in the last decade, the origin of the soil samples was mostly natural or controlled composting (18.5%), followed by agriculture (15.6%) and aquatic environments (14.5%). Forests and environments associated with them, e.g., groves (13.9%), were also moderately studied. The lowest studied environments included contaminated areas (11.0%), wetlands/coastal areas (10.4%), arid/semiarid areas (6.9%), industrial sludges (5.2%), mountainous areas (2.3%) and, finally, glacial areas (1.7%), probably due to the difficulty in accessing them.

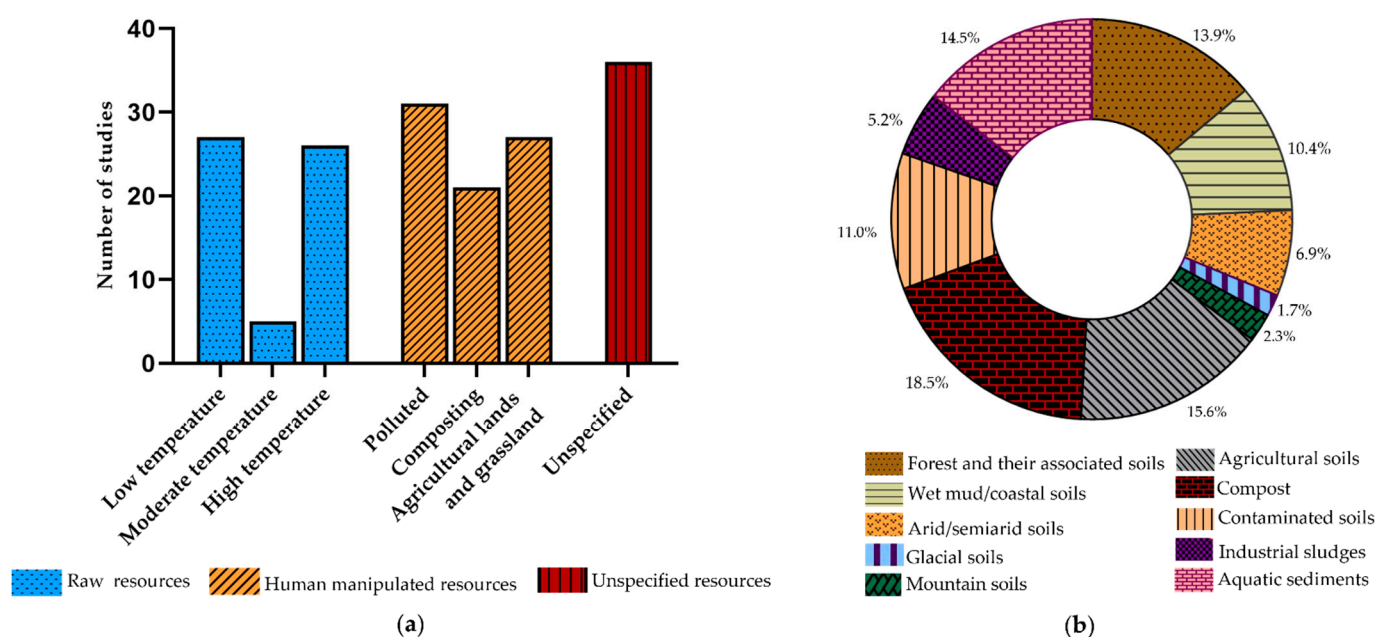


Figure 3. Classification of soil sampling sources explored to find promising biocatalysts through metagenomic approaches in the last decade. (a) Representation of the number of studies according to the defined categories (raw resources, human manipulated resources and unspecified resources). (b) Assignment of the soil type to each collected sample from different environments (forest and their associated soils, wet mud/coastal soils, arid/semiarid soils, glacial soils, mountain soils, agricultural soils, compost, contaminated soils, industrial sludge and aquatic sediments).

2.1. Raw Resources

Depending on the geographical location, the soil may present itself in various forms at the Earth's surface as the result of environmental conditions, organic matter content and type of vegetation [15]. An abiotic factor with high importance in any ecosystem is temperature since it can directly or indirectly affect microbial activity and, consequently, the composition of microbial communities. Each microbial species has a different temperature tolerance range and is capable of producing distinct types of biocatalysts at different production rates. Still, certain microorganisms can thrive and function well metabolically in adverse conditions, notably at extreme temperatures [16,17].

Therefore, the sampling locations in which the soil was directly collected and evaluated "as found in the nature" (raw resources) were classified according to the recorded temperature: low temperature (location temperature below 20 °C); moderate temperature (location temperature between 20 and 45 °C); and high temperature (location temperature above 45 °C) [17].

As shown in Figure 3a, a greater number of studies were accomplished in low-temperature environments (27 studies), followed by high-temperature environments (26 studies) and, finally, moderate temperature environments (5 studies). The unique characteristics of extreme environments, namely, extreme temperatures, make them more interesting places to find promising and robust enzymes. This fact is probably the main reason for the reduced number of studies accomplished in moderate temperature environments. Furthermore, there is an increasing need to find highly stable biocatalysts with efficient activities capable of acting in various industrial processes that make use of severe conditions, similar to what occurs in hot and cold environments [18]. Table 1 compiles all the data considered in the category of raw resources.

Table 1. Metagenomic studies reported in the last decade for soil samples classified in the category of raw resources.

Sample Source	Source Properties		Metagenomic Approach	Vector/ Host	No. Positive Clones/ Total Clones	Enzyme	No. Citations *	Reference
	pH	T (°C)						
Astaka region, Kargil, Northwestern Himalayas (India)	-	-	Functional	Cosmid/ <i>E. coli</i>	1/35,000	Amylase	42	[19]
Deep-sea, South China Sea (Pacific Ocean)	-	-	Functional	Fosmid/ <i>E. coli</i>	1/20,000	α -amylase	32	[20]
Ikaite tufa columns, Ikka Fjord (Greenland)	10.4	4.0	Sequence-based and functional	BAC/ <i>E. coli</i>	3/2843 2/2843	α -amylase β -galactosidases	70	[21]
Hot sulfur springs, Nubra Valley, Leh, Northern Himalayas (India)	7.5–8.5	60.0–80.0	Functional	Plasmid/ <i>E. coli</i>	1/10,000	β -D-galactosidase	24	[22]
Mountain of Flames, Turpan Basin, Xinjiang Uygur Autonomous Region (China)	-	76.0	Functional	Plasmid/ <i>E. coli</i>	1/8000	β -galactosidase	21	[23]
Composting, EXPO Park, Osaka (Japan)	7.5	67.0	Functional	Fosmid/ <i>E. coli</i>	10/6000	Cellulase	15	[24]
Farm-made composting, Khon Kaen (Thailand)	4.2–6.8	30.0–50.0	Functional	Fosmid/ <i>E. coli</i>	10/251	Endocellulase	13	[25]
Composting, Tainan (Taiwan)	-	-	Functional	Lambda phage vector/ <i>E. coli</i>	1/2739	Endoglucanase	30	[26]
Hydrothermal vent, Vulcano island (Italy)	5.9	100.0	Sequence-based	-	-	Endo- β -glucanase	14	[27]
Caldeirão hot spring, Furnas Valley, São Miguel Island, Azores (Portugal)	6.0–7.0	60.0–70.0	Functional	Plasmid/ <i>E. coli</i>	-	β -glucosidase	70	[28]
Turpan Basin, Xinjiang Uygur Autonomous Region (China)	-	82.0	Functional	Plasmid/ <i>E. coli</i>	5/50,000	β -glucosidase	68	[29]
Forest, National Institute of Advanced Industrial Science and Technology, Tsukuba (Japan)	-	15.0	Functional	Fosmid/ <i>E. coli</i>	1/50,000	β -glycosidase	22	[30]
Suruga Bay, Shizuoka, Japan (Pacific Ocean)	-	-	Sequence-based	-	-	β -glucosidases Endomannanase Endoxylanases β -xylosidases	17	[31]
Tattapani thermal spring, Chhattisgarh (India)	7.5–8.3	55.0–98.0	Sequence-based	-	-	β -glucosidase Xylanase	24	[32]
Thermophilic composting, Viçosa and Urucânia, Minas Gerais (Brazil)	-	55.0–65.0	Functional	Fosmid/ <i>E. coli</i>	159/6720 9/6720 14/6720	Cellulase Xylanase β -glucosidase	11	[33]

Table 1. Cont.

Sample Source	Source Properties		Metagenomic Approach	Vector/ Host	No. Positive Clones/ Total Clones	Enzyme	No. Citations *	Reference
	pH	T (°C)						
Solfatara-Pisciarelli hydrothermal pool, Agnano, Naples (Italy)	1.5	92.0	Sequence-based	-	-	β -mannanase/ β -1,3-glucosidase β -N-acetylglucosaminidase/ β -glucosidase	11	[34]
Hot spring compost-soil, Fukuoka (Japan)	3.0–4.5	-	Functional	Plasmid/ <i>E. coli</i>	-	Xylanase	73	[35]
Lobios hot spring, Ourense, Galicia (Spain)	5.9	76.0	Sequence-based and functional	Fosmid/ <i>E. coli</i>	1/150,000	Xylanase	15	[36]
Forest, National Institute of Advanced Industrial Science and Technology, Tsukuba (Japan)	-	15.0	Functional	Fosmid/ <i>E. coli</i>	1/50,000	α -xylosidase	21	[37]
Haiyan wetland, Qinghai-Tibetan Plateau (China)	-	-	Sequence-based	-	-	Chitinase	1	[38]
Cold desert, McMurdo Dry Valleys, South Victoria Land (Antarctica)	-	3.0	Functional	Fosmid/ <i>E. coli</i>	-/10,000	Esterase	38	[39]
Lake Arreo, Álava (Spain)	8.0	6.9	Functional	Fosmid/ <i>E. coli</i>	10/11,520	Esterases/lipases	39	[40]
Apharwat mountain, Jammu and Kashmir, North-western Himalayas (India)	-	-	Functional	Plasmid/ <i>E. coli</i>	1/10,000	Thioesterase	1	[41]
Composting, EXPO Park, Osaka (Japan)	7.5	67.0	Functional	Fosmid/ <i>E. coli</i>	19/6000	Esterase	9	[42]
Hot spring, Furnas Valley, São Miguel Island, Azores (Portugal)	2.0–7.0	60.0–62.0	Sequence-based and functional	Fosmid/ <i>E. coli</i> Fosmid/ <i>T. thermophilus</i>	1/6048 5/6048	Esterase	50	[43]
Apharwat mountain, Jammu and Kashmir, North-western Himalayas (India)	-	-	Functional	Plasmid/ <i>E. coli</i>	3/10,000	Esterase	3	[44]
Karuola glacier, Tibetan Plateau (China)	-	-	Functional	Fosmid/ <i>E. coli</i>	5/10,000	Esterase	10	[45]
Solnechny hot spring, Uzon Caldera, Kronotsky reserve, Kamchatka (Russia)	5.8–6.0	61.0–64.0	Sequence-based	-	-	Esterase	39	[46]
Permafrost, Kolyma Lowland, North-eastern Siberia (Russia)	-	-	Functional	Fosmid/ <i>E. coli</i>	7/5000	Esterase	30	[47]
Deep-sea, Barents Sea (Arctic Ocean)	-	-	Functional	Fosmid/ <i>E. coli</i>	19/3884	Esterase	24	[48]
Deep-sea, Svalbard e Jan Mayen (Arctic Ocean)	-	-	Functional	Fosmid/ <i>E. coli</i>	19/3884	Esterase		
Yellow Sea, China (Pacific Ocean)	-	-	Functional	Fosmid/ <i>E. coli</i>	34/40,000	Esterase	42	[49]
Acidic pool, Vulcano island (Italy)	-	≈25.0	Functional	Fosmid/ <i>E. coli</i>	44/1920	Carboxylesterases	44	[50]

Table 1. Cont.

Sample Source	Source Properties		Metagenomic Approach	Vector/ Host	No. Positive Clones/ Total Clones	Enzyme	No. Citations *	Reference
	pH	T (°C)						
Deep-sea (Atlantic Ocean)	-	-	Functional	Fosmid/ <i>E. coli</i>	6/17,000	Esterase	7	[51]
Deep-sea (Atlantic Ocean)	-	-	Functional	Fosmid/ <i>E. coli</i>	6/17,000	Esterase		
Solar saltern, Ribandar, Goa (India)	7.5	35.0	Functional	Fosmid/ <i>E. coli</i>	1/5100	Esterase	14	[52]
Qiongdongnan Basin, South China Sea (Pacific Ocean)	-	-	Functional	Fosmid/ <i>E. coli</i>	19/40,000	Esterase	68	[53]
Qiongdongnan Basin, South China Sea (Pacific Ocean)	-	-	Functional	Fosmid/ <i>E. coli</i>	1/200,000	Esterase	52	[54]
Deep-sea, South China Sea (Pacific Ocean)	-	-	Functional	Plasmid/ <i>E. coli</i>	15/60,000	Esterase	43	[55]
Kongsfjorden seashore, Ny-Alesund, Svalbard Archipelago (Norway)	-	-	Functional	Fosmid/ <i>E. coli</i>	2/60,000	Esterase	61	[56]
Turpan Basin, Xinjiang Uygur Autonomous Region (China)	-	82.0	Functional	Plasmid/ <i>E. coli</i>	3/21,000	Pyrethroid-hydrolysing esterase	49	[57]
Deep-Sea (Pacific Ocean)	-	1.5	Functional	Fosmid/ <i>E. coli</i>	12/20,000	Esterase	43	[58]
Composting, EXPO Park, Osaka (Japan)	7.5	67.0	Functional	Fosmid/ <i>E. coli</i>	19/6000	Cutinase	180	[59]
Taptapani hot spring, Odisha (India)	8.5	50.0	Functional	Plasmid/ <i>E. coli</i>	7/13,298	Lipase	21	[60]
Taptapani hot spring, Odisha (India)	8.5	50.0	Functional	Plasmid/ <i>E. coli</i>	7/13,000	Lipase	4	[61]
Qiongdongnan Basin, South China Sea (Pacific Ocean)	-	-	Functional	Plasmid/ <i>E. coli</i>	1/60,000	Laccase	28	[62]
Hot spring, Jiuqu, Guangxi (China)	-	65.0–68.0	Sequence-based	-	-	Aldehyde dehydrogenase	6	[63]
Ikahama hot spring, Tonami, Toyama (Japan)	-	-	Sequence-based	Plasmid/ <i>E. coli</i>	240/2800	Alcohol dehydrogenases	18	[64]
Farm composting, Toyama (Japan)	-	35.0–45.0	Sequence-based and functional	Plasmid/ <i>E. coli</i>	1200/2000	Alcohol dehydrogenases	15	[65]
Death Valley desert dune, California (USA)	-	-	Functional	Plasmid/ <i>E. coli</i>	1/30,000	Serine proteases	48	[66]
Gobi Desert dune, Ulaanbadrakh (Mongolia)	-	-	Functional	Fosmid/ <i>E. coli</i>	16/17,000			
Chumathang hot spring, Ladakh region, North-western Himalayas (India)	-	-	Functional	Plasmid/ <i>E. coli</i>	1/9000	Protease	10	[67]
Creek soil, Ardley Island, FILDES Peninsula (Antarctica)	-	-	Functional	Fosmid/ <i>E. coli</i>	7/-	Protease	0	[68]
Composting, EXPO Park, Osaka (Japan)	7.0	50.0	Functional	Plasmid/ <i>E. coli</i>	17/300,000	RNase H	10	[69]

Table 1. Cont.

Sample Source	Source Properties		Metagenomic Approach	Vector/ Host	No. Positive Clones/ Total Clones	Enzyme	No. Citations *	Reference
	pH	T (°C)						
Ladakh region, North-western Himalayas (India)	-	-	Functional	Plasmid/ <i>E. coli</i>	1/8500	Rhodanase	4	[70]
Caatinga biome, João Câmara, Rio Grande do Norte (Brazil)	-	21.0–32.0	Functional	Plasmid/ <i>E. coli</i>	1/2688	Exonuclease	12	[71]
Deep-sea (Arctic Ocean)	-	-	Sequence-based	Plasmid/ <i>E. coli</i>	1/2750	Chitin deacetylase	11	[72]
Tattapani thermal spring, Chhattisgarh (India)	7.7	98.0	Sequence-based	-	-	Trehalose synthase	0	[73]

* Obtained from the international database Scopus (21 March 2022).

2.1.1. Low-Temperature Environments

Cold soils, in addition to being exposed to very low temperatures, are also subject to other harsh conditions, such as freeze-thaw cycles, UV radiation and restricted availability of water and nutrients. Indeed, arid/semiarid regions, aquatic environments, polar regions, mountainous and forest areas are examples of cold habitats that have been the focus of metagenomic studies. Although they usually have low biodiversity, the microorganisms that inhabit them have acquired survival machinery and, for this reason, these types of habitats constitute stimulating reservoirs of biotechnological molecules [74].

Deep-sea sediments, mainly sands and clays from several aquatic environments, are the type of samples that most contribute to the exploration of enzymes in these cold habitats. The South China Sea is a marginal sea and one of the most studied environments since it constitutes an important reservoir of sediments rich in organic materials [75]. Other regions of the Pacific Ocean, such as Suruga Bay in Japan [31], as well as the depths of the Atlantic Ocean [51] and the Arctic Ocean, notably in the Barents Sea area [48], have also been studied to search not only for lipolytic enzymes but also enzymes belonging to the glycosyl hydrolases class. Additionally, the submarine tufa columns of the Ikka Fjord in Greenland [21] and the karstic lake in Spain (Lake Arreo) [40], which, in addition to being permanently cold, are alkaline, and valuable sources of microorganisms adapted to these environments. In addition to aquatic sediments, other types of samples with metagenomic exploitation potential come from arid/semiarid and mountain soils, for example in the Ladakh region [70] and the Agharwat mountain [41,44], respectively, both in the north-western Himalayas that have distinct geo-climatic characteristics like extremely cold and dry weather, high altitude and glacial and permafrost soils. Relevant examples of this are the Karuola glacier in Tibetan Plateau [45] and the Kolyma Lowland permafrost in north-eastern Siberia [47], which are extremely hostile environments and inhabited by unique microbial communities.

2.1.2. Moderate Temperature Environments

There are few studies in which sampling sources are identified as moderate temperature environments. Still, certain environments of this nature have other characteristics that also make them interesting for metagenomic purposes. An example is the Caatinga biome of João Câmara (Brazil) which presents sandy loam soil and constitutes an ecosystem of high biological relevance due to the features of the area, such as the semiarid climate, the high exposure to UV radiation and the long periods of drought [71,76]. Another example is the solar saltern of Goa, which differs by its high salinity and represents an important source for metagenomic studies given the difficulty in cultivating halophilic microorganisms through conventional techniques [52].

2.1.3. High-Temperature Environments

High-temperature environments have also proven to be important sources of very useful thermostable enzymes with applications in various industrial fields, such as food and chemical synthesis industries. In addition to geothermally heated environments, such as hot springs and hydrothermal vents, arid/semiarid regions and environments subject to natural composting processes are often good targets for the application of metagenomic tools [77]. Compost samples from Expo Park in Japan, produced from leaves and branches, are a good example of natural composting since they have been studied over a few years [24,42,59,69]. Once thermophilic composting reaches high temperatures, there is a greater predominance of microorganisms capable of degrading complex molecules, with this type of environment being a potential source of lignocellulose-degrading enzymes and, for this reason, an interesting subject of study [78]. Several arid/semiarid regions have been explored given the typical characteristics of these environments, including deserts [66] and also other sites—more specifically, the Turpan Basin, which represents China's hottest place and has proven to be a valuable source of different types of highly thermostable enzymes [57]. Hot springs and hydrothermal vents from different portions of the planet, e.g., Caldeirão hot spring in

Portugal [28], Solnechny hot spring in Russia [46] and Solfatara-Pisciarelli hydrothermal pool in Italy [34] have also contributed to finding robust enzymes through the construction of metagenomic libraries using DNA extracted from wet mud and/or sediments collected from these places. For all other metagenomic studies, the expression host used was *E. coli*, except for the metagenomic library constructed from sediments of a hot spring in the Azores, Portugal, which used the *T. thermophilus* as the host. Using this thermophilic host, Leis and co-workers intended to increase the probability of detecting genes derived from extreme environments that would encode for new thermostable biocatalysts and allow the screening of phenotypes that are not observable in *E. coli* [43].

In some studies performed from these raw resources, an additional enrichment step was performed to provide favourable growth conditions for certain microorganisms of interest, often present in small abundance, to the detriment of others [79]. These enrichments were implemented by introducing specific substrates, such as cellulose, xylan, chitin, starch and glucose [31,33,38], and even olive oil [47], that stimulate specific microbial activities. On the other hand, culture enrichment also occurred by controlling environmental conditions, in particular the temperature, which is generally in agreement with the temperature of the sampling locations [26].

Over the past decade, in addition to function-based metagenomic screenings, sequence-based metagenomic screenings have also been performed. Sequential metagenomics showed that the phyla that predominate high-temperature environments are *Crenarchaeota*, *Thaumarchaeota*, *Acidobacteria* and *Proteobacteria* capable of mineral-based metabolism and generally associated with soil, found more specifically in sediments from hot springs and hydrothermal vents [21,31,34,36,43].

2.2. Human Manipulated Resources

Soil, in addition to being “discovered” as it is exposed in nature, without any kind of alteration, can be studied in a variety of scenarios, including contaminated/polluted, agricultural and controlled composting as a consequence of human manipulations. According to Figure 3a, 31 metagenomic studies were performed in polluted soil samples, 27 studies were conducted in agricultural fields and grasslands and 21 studies were performed on samples provided by composting facilities. Table 2 comprises all the data considered in the category of human manipulated resources.

Table 2. Metagenomic studies reported in the last decade for soil samples classified in the category of human manipulated resources.

Sample Source	Source Properties		Metagenomic Approach	Vector/ Host	No. Positive Clones/ Total Clones	Enzyme	No. Citations *	Reference
	pH	T (°C)						
Daqing oil field, Heilongjiang (China)	-	-	Functional	Plasmid/ <i>E. coli</i>	3/12,000	β -galactosidase	47	[80]
Agricultural corn field, Elora, Ontario (Canada)	7.8	-	Functional	Cosmid/ <i>E. coli</i>	161/79,060	β -galactosidase	28	[81]
Grover Soil Solutions compost facility, California (USA)	-	-	Sequence-based	-	-	Cellulase	139	[82]
Sugarcane land field, São Carlos (Brazil)	-	-	Functional	Plasmid/ <i>E. coli</i>	1/26,900	Cellulase	43	[83]
Pollutants- contaminated stream ground surface, Guangxi (China)	9.5	-	Functional	Plasmid/ <i>E. coli</i>	2/30,000	β -glucosidases	40	[84]
Yingtian Red Soil Ecological Station, Jiangxi (South China)	-	-	Functional	Plasmid/ <i>E. coli</i>	1/3024	Endo- β -1,4-glucanase	54	[85]
Straw stook, Jiangxia, Wuhan (China)	-	-	Functional	Plasmid/ <i>E. coli</i>	1/24,000	Endoglucanase	12	[86]
Agricultural fields irrigated with effluents of paper and pulp mill, Uttarakhand (India)	-	-	Functional	Fosmid/ <i>E. coli</i>	1/7500	β -1,4-endoglucanase	6	[87]
Shek Wu Hui Sewage Treatment Works, Hong Kong (China)	-	55.0	Sequence-based	-	-	Endo- β -1,4-glucanases	50	[88]
Composting, State University of Paraíba composting cells, Campina Grande (Brazil)	-	55.0–70.0	Functional	Cosmid/ <i>E. coli</i>	1/10,000	Endoglucanase	16	[89]
Sugarcane land field, São Carlos (Brazil)	-	-	Functional	Plasmid/ <i>E. coli</i>	1/26,900	Endoglucanase	15	[90]
Yonghyeon Nonghyup compost factory, Sacheon (South Korea)	8.9–9.2	40.0–73.0	Functional	Fosmid/ <i>E. coli</i>	2/12,380	Endo- β -1,4-glucanases	10	[91]
Grassland rhizosphere, Teagasc Oak Park research facility, Carlow (Ireland)	-	-	Functional	Fosmid/ <i>E. coli</i>	1/45,000	Endo- β -1,4-glucanase	14	[92]
Municipal compost platform, Bailly (France)	-	50.0	Functional	Fosmid/ <i>E. coli</i>	74/48,000	Endoglucanases	2	[93]
Paddy soil, Liaoning (China)	-	-	Functional	Fosmid/ <i>E. coli</i>	-/25,000	β -glucanase	26	[94]
University of Agricultural Sciences field, Uppsala (Sweden)	6.9	-	Sequence-based	Fosmid/ <i>E. coli</i>	-/7800	Chitinase	64	[95]
University of Agricultural Sciences field, Uppsala (Sweden)	6.9	-	Functional	Fosmid/ <i>E. coli</i>	1/7800	Chitinase	57	[96]
Chitin-treated agricultural field, Experimental farm “Vredepeel” (The Netherlands)	5.7	-	Sequence-based and functional	Fosmid/ <i>E. coli</i>	5/145,000	Chitinase	17	[97]

Table 2. Cont.

Sample Source	Source Properties		Metagenomic Approach	Vector/ Host	No. Positive Clones/ Total Clones	Enzyme	No. Citations *	Reference
	pH	T (°C)						
Chitin- contaminated soil, Mahtani Chitosan, Gujarat (India)	-	-	Sequence-based	-	-	Chitinase	13	[98]
Fat-contaminated soil, industrial wastewater treatment plant lagoon, Paraná (Brazil)	-	30.0	Functional	Fosmid/ <i>E. coli</i>	15/500,000	Chitinase	15	[99]
Carnoulès acid- mine drainage, Gard (France)	3.8	15.1	Functional	Plasmid/ <i>E. coli</i>	28/80,000	Amylases	16	[100]
Oil-contaminated mangrove site, Bertioga, São Paulo (Brazil)	-	-	Functional	Fosmid/ <i>E. coli</i>	1/12,960	β -N-acetylhexosaminidase	8	[101]
Yonghyeon Nonghyup compost factory, Sacheon (South Korea)	8.9–9.2	40.0–73.0	Functional	Fosmid/ <i>E. coli</i>	5/12,380	Xylanase	30	[102]
Sugarcane land field, São Carlos (Brazil)	-	-	Functional	Plasmid/ <i>E. coli</i>	1/26,900	Endoxylanase	20	[103]
Ämmässuo composting plant (Finland)	-	-	Functional	Fosmid/ <i>E. coli</i> Plasmid/ <i>E. coli</i>	21/43,000 18/40,000 1/760,000	Xylanases	8	[104]
Jiaozuo Ruifeng Paper Co., Ltd., Jiaozuo, Henan province (China)	-	-	Sequence-based	-	-	Xylanase	2	[105]
Commercial compost production facility, Western Cape (South Africa)	-	70.0	Functional	Fosmid/ <i>E. coli</i>	26/20,000	β -xylosidase	0	[106]
Yonghyeon Nonghyup compost factory, Sacheon (South Korea)	8.9–9.2	40.0–73.0	Functional	Fosmid/ <i>E. coli</i>	2/12,380 5/12,380	Cellulase Xylanases	16	[107]
Grover Soil Solutions and the Jepson Prairie Organics compost factories, California (USA)	-	-	Sequence-based	-	-	Endoglucanase Xylanase	76	[108]
Grover Soil Solutions compost facility, California (USA)	-	-	Sequence- based	-	-	β -xylosidase/ α -arabinofuranosidase Endoxylanases α -fucosidase	41	[109]
Industrial bagasse collection site, Phu Khieo Bio-Energy, Chaiyapoom (Thailand)	-	49.0–52.0	Functional	Fosmid/ <i>E. coli</i>	7/100,000	Endoglucanase Endoxylanase	34	[110]
Oil- contaminated soils, Ile des Petrels, Terre Adélie (Antarctica)	-	12.0	Functional	BAC/ <i>E. coli</i>	14/113,742 14/113,742 3/113,742 11/113,742	Lipases/esterases Amylases Proteases Cellulases	32	[111]

Table 2. Cont.

Sample Source	Source Properties		Metagenomic Approach	Vector/ Host	No. Positive Clones/ Total Clones	Enzyme	No. Citations *	Reference
	pH	T (°C)						
Experimental field luvisoil, Gembloux (Belgium)	6.5–7.0	-	Functional	Yeast episomal shuttle vector/ <i>E. coli</i>	7/500,000 2/500,000	β -glycosidases Glycosyltransferases	7	[112]
Swine wastewater treatment facility, Tainan (Taiwan)	7.6	30.0	Sequence-based and functional	Plasmid/ <i>E. coli</i>	13/3818	Esterases	37	[113]
Yonghyeon Nonghyup compost factory, Sacheon (South Korea)	8.9–9.2	40.0–73.0	Functional	Fosmid/ <i>E. coli</i>	19/23,400	Esterase	52	[114]
Plants rhizosphere, Gyeongsang (South Korea)	-	-	Functional	Fosmid/ <i>E. coli</i>	14/142,900	Esterase	42	[115]
Wheat field, Shouguang, Shandong (China)	-	-	Functional	Plasmid/ <i>E. coli</i>	6/50,000	Feruloyl esterase	18	[116]
Petroleum hydrocarbons- contaminated region, Ribeirão Preto, São Paulo (Brazil)	-	-	Functional	Fosmid/ <i>E. coli</i>	30/4224	Esterase	21	[117]
Commercial compost production facility, Western Cape (South Africa)	6.1	70.0	Sequence-based and functional	Fosmid/ <i>E. coli</i>	25/110,592	Feruloyl esterase	12	[118]
Bioenergiezentrum GmbH compost facility, Göttingen (Germany)	-	63.3	Sequence-based and functional	Fosmid/ <i>E. coli</i> Fosmid/ <i>T. thermophilus</i>	1/1920 1/1920	Esterase	50	[43]
Yonghyeon Nonghyup compost factory, Sacheon (South Korea)	8.9–9.2	40.0–73.0	Functional	Fosmid/ <i>E. coli</i>	18/23,400	Esterase	28	[119]
Tembec Paper Mill, Temiscaming, Ontario (Canada)	-	25.0–35.0		Plasmid/ <i>E. coli</i>	1/53,500			
Waste water treatment plant anaerobic digester, Evry (France)	-	33.0		Fosmid/ <i>E. coli</i>	254/47,616			
Oil- contaminated harbour, Priolo Gargallo (Italy)	-	15.0	Functional		4/118,500	Carboxylesterases	44	[50]
PAH contaminated soil, Michle (Czech Republic)	-	20.0–25.0			20/99,900			
PAH contaminated bioremediation site, Sobeslav (Czech Republic)	-	-		Plasmid/ <i>E. coli</i>	5/114,000			
Composting plant, Liemehna (Germany)	-	30.0–50.0			6/60,000			
Biodiversity Exploratories Schorfheide-Chorin forest and grassland site (Germany)	-	8.0–8.5	Functional	Plasmid/ <i>E. coli</i> Fosmid/ <i>E. coli</i>	28/40,000–341,000 9/4600–300,000	Esterase	15	[120]
Bioenergiezentrum GmbH compost facility, Göttingen (Germany)	-	55.0	Functional	Plasmid/ <i>E. coli</i>	279/675,200	Esterases	6	[121]

Table 2. Cont.

Sample Source	Source Properties		Metagenomic Approach	Vector/Host	No. Positive Clones/ Total Clones	Enzyme	No. Citations *	Reference
	pH	T (°C)						
Cotton field (China)	-	-	Functional	Plasmid/ <i>E. coli</i>	1/92,000	Esterase	0	[122]
Cornfield, Shangqiu, Henan province (China)	-	-	Functional	Fosmid/ <i>E. coli</i>	1/30,000	Carboxylesterase	1	[123]
Waste contaminated farmland, Nanjing, Jiangsu province (China)	-	-	Functional	Fosmid/ <i>E. coli</i>	9/60,000	Carboxylesterase	2	[124]
Yonghyeon Nonghyup compost factory, Sacheon (South Korea)	8.9–9.2	40.0–73.0	Functional	Fosmid/ <i>E. coli</i>	19/23,400	Esterase	0	[125]
Yonghyeon Nonghyup compost factory, Sacheon (South Korea)	8.9–9.2	40.0–73.0	Functional	Fosmid/ <i>E. coli</i>	19/23,400	Esterases	1	[126]
Oil-contaminated Mud Flat, Taean (South Korea)	-	-	Functional	Plasmid/ <i>E. coli</i>	8/3000	Esterases	2	[127]
Teagasc Oak Park research facility organic field trial site, Carlow (Ireland)	-	-	Functional	Fosmid/ <i>E. coli</i>	1/14,000	Esterase/lipase	9	[128]
Fat-contaminated soil, Paraná (Brazil)	-	30.0	Functional	Fosmid/ <i>E. coli</i>	32/500,000	Lipase	129	[129]
Oil-contaminated soil, Qingshan Branch oil field, Anhui (China)	-	-	Functional	Plasmid/ <i>E. coli</i>	6/20,000	Lipase	17	[130]
Oil-contaminated mud flat, Taean (South Korea)	-	-	Functional	Plasmid/ <i>E. coli</i>	9/3000	Lipase	25	[131]
Cotton field (China)	-	-	Functional	Plasmid/ <i>E. coli</i>	1/92,000	Tannase	28	[132]
Biodiversity Exploratories Hainich-Dün forest and grassland site (Germany)	6.5–8.0							
Biodiversity Exploratories Schwäbische Alb forest and grassland site (Germany)	6.0–7.0	-	Functional	Plasmid/ <i>E. coli</i> Fosmid/ <i>E. coli</i>	28/40,000–341,000 9/4600–300,000	Lipolytic enzymes	54	[133]
Biodiversity Exploratories Schorfheide-Chorin forest and grassland site (Germany)	8.0–8.5							
Experimental field luvisoil, Gembloux (Belgium)	6.5–7.0	-	Functional	Yeast episomal shuttle vector/ <i>E. coli</i>	19/420,000	Lipolytic enzymes	1	[134]
Wastewater treatment facility (Japan)	-	-	Functional	Fosmid/ <i>E. coli</i>	1/100,000	Bilirubin oxidase	8	[135]
Contaminated rice field, Nanning, Guangxi (China)	-	-	Sequence-based	Plasmid/ <i>E. coli</i>	1/32,000	D-Amino acid oxidase	7	[136]
Coke plant wastewater treatment facility (Japan)	-	-	Functional	Fosmid/ <i>E. coli</i>	6/40,000	Oxygenases	19	[137]
Pesticide industry effluent treatment plant (India)	-	-	Functional	Plasmid/ <i>E. coli</i>	2/40,000	Flavin monooxygenases	22	[138]

Table 2. Cont.

Sample Source	Source Properties		Metagenomic Approach	Vector/ Host	No. Positive Clones/ Total Clones	Enzyme	No. Citations *	Reference
	pH	T (°C)						
PAH-contaminated wetland, Chambéry (France)	-	-	Sequence-based	-	-	Dioxygenases	34	[139]
Polychlorinated biphenyl-contaminated, Fushun, Liaoning (China)	8.3	-	Functional	Plasmid/ <i>E. coli</i>	1/-	2,4-dichlorophenol hydroxylase	23	[140]
Compost-producing company, Toyama (Japan)	-	50.0–80.0	Sequence-based and functional	Plasmid/ <i>E. coli</i>	1200/2000	Alcohol dehydrogenases	15	[65]
Farm, Knockbeg (Ireland)	-	-	Functional	Fosmid/ <i>E. coli</i>	28/14,400	Phytases	14	[141]
Paddy field, Yuseong, Daejeon (South Korea)	-	-	Functional	Fosmid/ <i>E. coli</i>	157/326,200	Wax ester synthase	6	[142]
Plants rhizosphere, Gyeongsang (South Korea)	-	-	Functional	Fosmid/ <i>E. coli</i>	157/326,200	Wax ester synthase	6	[142]
Lop Nur, Xinjiang Uigur Autonomous Region (China)	-	-	Functional	Plasmid/ <i>E. coli</i>	1/85,000	Trehalose synthase	34	[143]
Cornfield, Turpan Basin, Xinjiang Uygur Autonomous Region (China)	-	-	Functional	Plasmid/ <i>E. coli</i>	1/700,000	Glycosyltransferase	14	[144]
Municipal sewage plant, Garmerwolde (The Netherlands)	6.4	-	Functional	Plasmid/ <i>E. coli</i>	6/1,250,000 8/1,250,000	Sulfatases Phosphotriesterases	146	[145]
Urban composting facility, Groningen (The Netherlands)	7.9	-	Functional	Plasmid/ <i>E. coli</i>	6/1,250,000 8/1,250,000	Sulfatases Phosphotriesterases	146	[145]
Local agricultural field (The Netherlands)	8.2	-	Functional	Plasmid/ <i>E. coli</i>	6/1,250,000 8/1,250,000	Sulfatases Phosphotriesterases	146	[145]

* Obtained from the international database Scopus (21 March 2022).

2.2.1. Polluted Environments

The intensification of industrialization, urbanization and mining have negatively affected the soil as a natural source. It has been observed that soil has been contaminated by different factors, namely, industrial sewages, solid wastes and urban activities. Some organic and inorganic pollutants have been responsible for soil contamination, such as heavy metals, alkaline or acidic constituents, toxins, oil contaminants and others [146].

It was found that the following categories of polluted samples have been used as the object of metagenomic studies: soils contaminated by oil and its constituents (such as polycyclic aromatic hydrocarbons (PAHs)), fertilizers and other alkaline pollutants, industrial sludges and sediments. Oil production sites [80], soils where oil spills or runoffs have occurred [50,101,111,139], soils near industrial areas [50,87,117,140] and soils treated with fertilizers [136] were particularly analyzed. Since pollutants are rich in toxic compounds, they affect the activity and diversity of microbial communities present in these adulterated soils. Therefore, metagenomic studies have been developed in this type of compromised environment to unravel the gene clusters that encode enzymes involved in the biodegradation of the various pollutants already mentioned. Only the microorganisms that transport machinery capable of resisting and degrading these types of recalcitrant compounds can survive in such environments. The toxic compounds can even act as substrates and enrich some specific microorganisms [147].

The acid mine drainage in Carnoulès (France) is considered an interesting reservoir of enzymes capable of degrading polymers and pollutants simultaneously producing antimicrobial agents, since this polyextreme environment, in addition to being highly acidic, presents high concentrations of heavy metals, such as iron and arsenic, as a consequence of mining [100]. Another example is the saline–alkali soil of Lop Nur (China) which is characterized by extreme aridity and is a location that suffers from severe human manipulation. Since it serves as a basis for monitoring and verification of nuclear tests [148], microorganisms present in this soil are certainly subject to a high degree of stress and, for this reason, it may present an interesting microbial diversity and functionality [143].

Other areas exposed to other components such as fats [99,129] or chitin [97,98] have also been the focus of metagenomic studies as they are potential sources of new genes encoding groups of specific enzymes (lipolytic and chitinolytic enzymes, respectively). Activated sludge from different municipal [50,88,135,145] or industrial effluents, such as pesticide [138], swine [113] and paper and pulp [50] industries, are also a rich source of microorganisms producing enzymes capable of degrading protein, lipids and other pollutants. Of the thirty-one studies, two of them performed the analysis of the 16S rRNA gene libraries constructed, one of activated sludge from a swine wastewater treatment facility and the other one from soil contaminated and enriched with chitin. In both studies, it was found that the most predominant phylum was *Proteobacteria* [98,113]. Nevertheless, different samples can unravel other dominant ones, since the composition of the sources (activated sludge from industrial or municipal wastewater treatment plants or treated soils) and the type of treatment accomplished may influence the bacterial diversity.

2.2.2. Agricultural Lands and Grassland

Land use and management have a great influence on the functioning of the soil ecosystem. Microbial diversity and functionality are sensitive to land use considering the important role of soil microorganisms in soil formation processes and nutrient cycling [149].

Hence, several metagenomic studies were implemented in fields designed for agriculture and/or grasslands, many of them subject to the ploughing and cultivation of different crops. Rhizosphere soils of, for example, red pepper plants and strawberry plants represent a complex but interesting ecosystem due to the symbiosis and parasitism interactions that happen between plants and microorganisms in these soil regions [92,115,142]. Cotton [132], wheat [116,141], sugarcane [83,90,103], corn [81,144], straw [86] and paddy [94,142] fields are examples of agricultural environments from which samples were collected, in particular from topsoil, to be analysed through metagenomic approaches. The selection of topsoil

samples and not samples at higher depths is due to the presence of a higher soil microbial biomass on the surface since there is larger evidence of litter composition and root turnover rates in this type of land [149]. Another important fact is that decomposition of a variety of lignocellulosic residues occurs in these environments making them attractive to isolate lignocellulose-degrading enzymes relevant to several industrial applications.

Three large-scale research landscapes [133] in Germany (Hainich-Dün, Schorfheide-Chorin and Schwäbische Alb) are defined as exploratory environments. They present different geological and climatic conditions and are characterized by the different intensities of use and management of agricultural fields and grasslands. Therefore, they certainly have great microbial diversity. In these environments, 37 novel lipolytic enzymes, the vast majority belonging to the hormone-sensitive lipase family, were reported (Table 2). These exploratory environments, together with the Oak Park research facility in Ireland [128], are valuable sources of promising biocatalysts, notably lipases/esterases, as their land is essentially fertilized with compost and/or manure and is subject to crop rotation. The crop rotation system benefits certain chemical and physical properties of the soil, which is very important and favourable for soil microorganisms [150].

2.2.3. Industrial Composting

Among the different manipulated sources, composting is considered one of the most important bio-reactions for renewable bioenergy on the planet due to the huge variety of microorganisms capable of degrading lignocellulosic biomass. Composting is a sustainable and efficient microbiological process in which the stabilization of the organic matter occurs due to the passage through a thermophilic phase promoted by the proliferation of thermophilic microorganisms [78,151].

The great contribution of composting to the circular economy has led to an increase in the number of composting facilities that are responsible for the production of compost, rich in humic substances, with high agronomic value in organic fertilization of agricultural soils. Different parameters are controlled and adjusted throughout the industrial composting process, such as temperature, pH, humidity, nature of organic materials, particle size and C/N ratio [151].

Some raw materials used in industrial composting are agricultural and agro-industrial residues, including animal faeces [91,102,107,114,118,119], household wastes [108,121], residues from crop harvesting [82,89,91,102,107,109,110,114,119], green wastes [82,104,108,109,118,121], wood chips and sawdust [43,118] and the organic putrescible fraction of municipal solid waste [93,145].

For the metagenomic studies in which composting samples were used, the sample collection essentially occurred during the thermophilic phase of composting that reaches high temperatures (above 45 °C) due to microbial metabolic activity. Certain studies refer to microbial diversity and confirm the prevalence of thermophilic microorganisms, namely, *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Proteobacteria*, producing enzymes able to degrade the complex molecules that compose the lignocellulosic biomass [43,82,108,118]. Additionally, some studies also report enrichment with lignocellulosic substrates, namely, switchgrass, steam-exploded spruce, cutter chips, Whatman filter paper, pre-treated *Miscanthus giganteus* and wheat straw. These substrates are firstly incubated with the composting samples at a constant temperature and pH-defined according to the phase at which they were collected to increase the abundance of thermophilic microorganisms and develop a suitable consortium able to degrade lignocellulosic biomass. Over the years, several lignocellulose degrading enzymes have been reported through the analysis of industrial composting metagenomes [82,93,104,108,109].

2.3. Unspecified Resources

The designation “unspecified” includes studies from soil resources that do not fall into either the raw resources section or the section of resources subject to human manipulation,

since neither the temperature of the sample collected, the sampling site nor the existence of any human activity which may alter the natural properties of the sample is mentioned.

A large part of the unspecified resources are forests that are made up of microorganisms responsible for mediating biogeochemical cycles in terrestrial ecosystems. Additionally, forest soils have a high microbial diversity due to the great accumulation of organic matter [15]. Several metagenome studies have been developed, notably in soil samples from the Amazon rainforest [152], mangrove forests [153–157], peat-swamp forests [158,159], beech forests [160] and chestnut groves [161], to assess microbial diversity, as well as the metabolic capacities of these communities to decompose natural biomass. A metagenome from the *Eucalyptus* sp. forest in Brazil revealed that two of the most abundant phyla are *Actinobacteria* and *Firmicutes*, commonly associated with forest soils [15,162].

This category also includes samples from soils with high moisture content, such as alluvial soils from Eulsukdo Island (South Korea) [142,163], soils collected from lakes [64,145] and even soils with a significant salinity content [145,164], mountain soils such as an acid peatland site in Germany [159] and arid areas, namely, soils of the Cerrado region of Brazil [165,166] that present a high clay content, low pH and high iron levels. All these environments have interesting characteristics that give them unique ecosystems with high enzymatic potential. Table 3 comprises all the data considered in the category of unspecified resources.

Table 3. Metagenomic studies reported in the last decade for soil samples classified in the category of unspecified resources.

Sample Source	Source Properties		Metagenomic Approach	Vector/ Host	No. Positive Clones/ Total Clones	Enzyme	No. Citations *	Reference
	pH	T (°C)						
Shenzhen Mangrove Reserve, Guangdong (China)	-	-	Functional	Plasmid/ <i>E. coli</i>	1/30,000	β -glucosidase	64	[155]
Amazon forest, Moju, Pará (Brazil)	5.5	-	Functional	Fosmid/ <i>E. coli</i>	5/97,500	β -glucosidase	18	[152]
<i>Eucalyptus</i> sp. forest, UNESP campus, São Paulo (Brazil)	-	-	Sequence-based	-	-	β -glucosidase	16	[162]
Mangrove Reserve, Sanya, Hainan (China)	-	-	Functional	Fosmid/ <i>E. coli</i>	1/100,000	Endo- β -1,4-glucanase	24	[156]
Junggar Basin, Xinjiang (China)	-	-	Functional	Plasmid/ <i>E. coli</i>	1/7200	Cellulase	4	[167]
Garden, Zwingenberg (Germany)	-	-	Functional	Plasmid/ <i>E. coli</i>	6/1,335,000	β -galactosidase	39	[168]
RGS 90 A/S environmental company, Copenhagen (Denmark)	-	-	Functional	Fosmid/ <i>E. coli</i>	7/100,000	α -fucosidase	43	[169]
Forest, Daejeon (South Korea)	-	-	Functional	Plasmid/ <i>E. coli</i>	1/19,626	Lichenase	6	[170]
Mangrove Reserve, Sanya, Hainan (China)	-	-	Functional	Fosmid/ <i>E. coli</i>	1/100,000	β -agarase	18	[157]
Sathyamangalam forest, Erode, Tamilnadu (India)	-	-	Functional	Plasmid/ <i>E. coli</i>	9/2000	Pectin degrading glycosyl hydrolase	13	[171]
Coastal saline area, Tianjin (China)	-	-	Sequence-based	Plasmid/ <i>E. coli</i>	12/-	Xylanase	12	[164]
Backyard, Kyushu University, Fukuoka (Japan)	-	-	Functional	Plasmid/ <i>E. coli</i>	5/150,000 1/150,000	Cellulase Xylanase	24	[172]
Forest, Yunnan (China)	-	-	Functional	Cosmid/ <i>E. coli</i>	1/150,000	Cellulase/ Hemicellulase	8	[173]
Mangrove forest, Pontal do Paraná (Brazil)	-	-	Functional	Fosmid/ <i>E. coli</i>	1/2400	Lipase	41	[153]
Atlantic forest, Paraná (Brazil)	3.7–4.4	-	Functional	Fosmid/ <i>E. coli</i>	1/34,560	Lipase	25	[174]
Cerrado <i>sensu stricto</i> area, Brazilian Institute of Geography and Statistics (Brazil)	4.7	-	Functional	Plasmid/ <i>E. coli</i>	3/6720	Lipase	3	[165]
Mt. Jumbong reed marsh, Gangwon province (South Korea)	-	-	Functional	Plasmid/ <i>E. coli</i>	46/112,500	Lipase	0	[175]
Sirinthon Peat-Swamp Forest, Narathiwat (Thailand)	5.0	-	Functional	Fosmid/ <i>E. coli</i>	6/15,000	Esterase	34	[158]
Alluvium, Eulsukdo Island, Saha-Gu, Busan (South Korea)	-	-	Functional	Fosmid/ <i>E. coli</i>	50/45,300	Esterases	20	[163]
Mount Fanjing, Tongren, Guizhou (China)	-	-	Functional	Fosmid/ <i>E. coli</i>	1/50,000	Esterase	35	[176]
Göttingen beech forest, Georg-August University, Göttingen (Germany)	-	-	Functional	Plasmid/ <i>E. coli</i>	3/70,000	Esterase	11	[160]

Table 3. Cont.

Sample Source	Source Properties		Metagenomic Approach	Vector/ Host	No. Positive Clones/ Total Clones	Enzyme	No. Citations *	Reference
	pH	T (°C)						
Forest, Groenendaal (Belgium)	-	-	Functional	Plasmid/ <i>E. coli</i>	3/70,000	Esterases	40	[177]
Chestnut grove surface, Yunnan (China)	-	-	Functional	Cosmid/ <i>E. coli</i>	2/100,000	Esterases	7	[161]
Tree rhizosphere, Korea Expressway Corporation Arboretum, Jeonju (South Korea)	-	-	Functional	Cosmid/ <i>E. coli</i>	1/7968	Carboxylesterase	10	[178]
Composting, Cheongyang (South Korea)	-	-	Functional	Fosmid/ <i>E. coli</i>	14/13,000	Carboxylesterase	4	[179]
Shenzhen Mangrove Reserve, Guangdong (China)	-	-	Functional	Plasmid/ <i>E. coli</i>	1/8000	Laccase	69	[154]
Lake Biwa, Otsu, Shiga (Japan)	-	-	Sequence-based	Plasmid/ <i>E. coli</i>	240/2800	Alcohol dehydrogenases	15	[65]
Masukata Park, Uozu, Toyama (Japan)	-	-						
Cerrado <i>sensu stricto</i> area, Brazilian Institute of Geography and Statistics (Brazil)	4.7	-	Functional	Plasmid/ <i>E. coli</i> Fosmid/ <i>E. coli</i>	3/150,000 3/65,000	Dioxygenase	12	[166]
Forest, Ehime Research Institute of Agriculture, Forestry, and Fisheries, Matsuyama (Japan)	-	-	Functional	Cosmid/ <i>E. coli</i>	29/208,000	Oxygenases	35	[180]
Schlöppnerbrunnen peatland site, Fichtelgebirge Mountains, Northeastern Bavaria (Germany)	4.0	-	Sequence-based	-	-	Phytase	10	[159]
Forest, Groenendaal (Belgium)	-	-	Functional	Plasmid/ <i>E. coli</i>	1/70,000	Serine protease	41	[181]
Saline flats surface, Paesens-Moddergat (The Netherlands)	8.0	-	Functional	Plasmid/ <i>E. coli</i>	6/1,250,000 8/1,250,000	Sulfatases Phosphotriesterases	146	[145]
Lauwersmeer lakeshore (The Netherlands)	8.5	-						
Alluvium, Eulsukdo Island, Saha-Gu, Busan (South Korea)	-	-	Functional	Fosmid/ <i>E. coli</i>	158/326,200	Wax ester synthase	6	[142]
Gwangneung forest, Korea National Arboretum, Gyeonggi (South Korea)	-	-						

* Obtained from the international database Scopus (21 March 2022).

3. Water

Water is essential in our life. Although frequently perceived as just an ordinary substance, it plays a vital role on the Earth since life as we know it could not exist without water. As a natural resource, water is fully distributed over the planet. The oceans contain the greatest fraction of water (96.5%), while continental water is composed of freshwater (2.5%) and saline groundwater (1%) [182]. Water is present in its three physical forms (liquid, solid and vapour) depending on the climate conditions. Aqueous environments (saline and freshwater) are considered important sources of microorganisms capable of inhabiting and resisting in different physical forms, such as icebergs (important spots for marine life), seas and oceans, thermal springs, glaciers, lakes and ponds. The microbial diversity and functionality found in water are strongly dependent on the environmental conditions (e.g., salinity, temperature, physical form, nutrients, pH or depth). It is known that a large part of the aquatic microbial resources remains unexplored mainly due to access limitations. Nevertheless, the use of metagenomic tools has significantly aided the discovery of novel biocatalysts from aquatic metagenomes [183]. In this review, the water samples used in the metagenomic studies were divided according to their origin (Figure 4a) and main characteristics (Figure 4b). In the last decade, a total of 26 metagenomic studies with water samples to find promising enzymes were reported (Figure 4a).

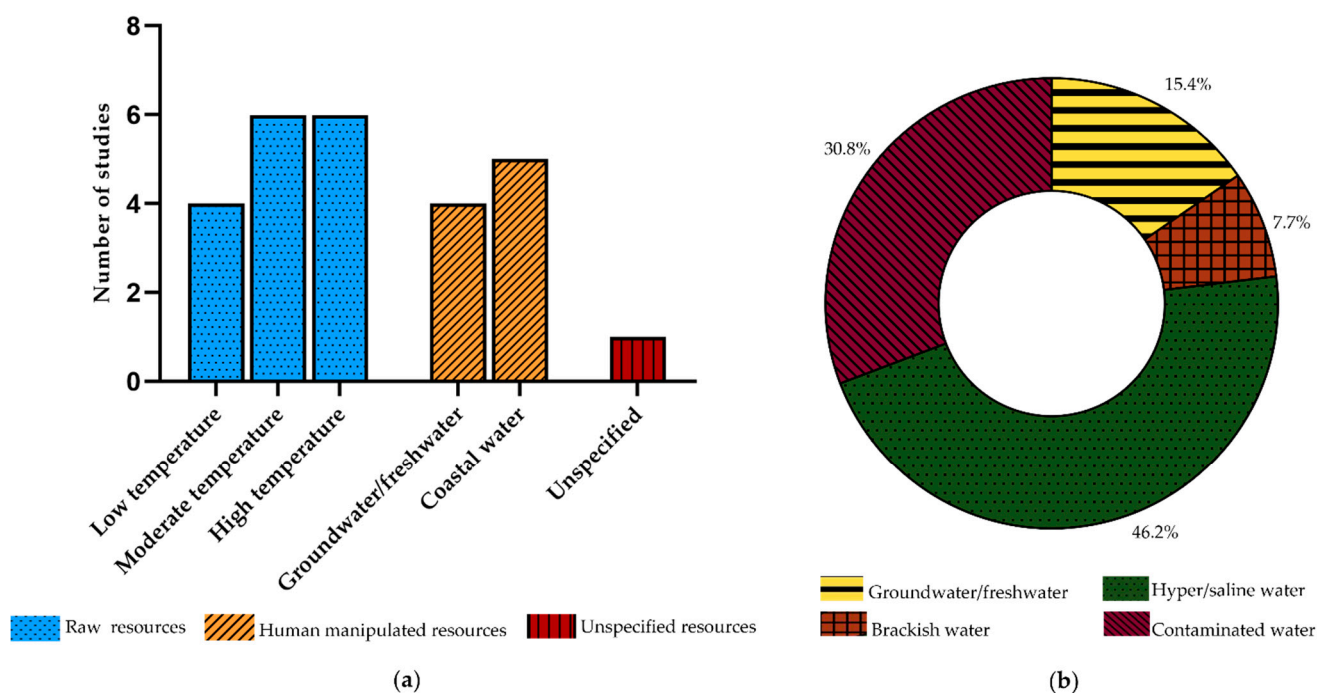


Figure 4. Classification attributed to water sampling sources explored to find promising biocatalysts through metagenomic approaches in the last decade. (a) Representation of the number of studies according to the categories defined (raw resources, human manipulated resources and unspecified resources). (b) Assignment of water type to each sample collected in different environments (groundwater/freshwater, brackish water, hyper/saline water and contaminated water).

As shown in Figure 4b, in the last decade the most studied aqueous samples were hyper/saline water (46.2%), followed by contaminated water (30.8%) and groundwater/freshwater (15.4%) and, finally, brackish water (7.7%). The first two types of water have been the most explored probably due to their extreme characteristics.

3.1. Raw Resources

As mentioned before, the microbial communities of natural water resources are strongly associated with environmental factors, one of which is temperature [184]. Indeed,

in addition to the most common aquatic environments on the planet, such as seawater or lakes, extreme temperature environments also allow exploring a diversity of enzymes capable of catalysing reactions at reasonable or extreme conditions [185]. Therefore, raw water resources were classified in the same way and according to the same temperature ranges as soil resources: low temperature (location temperature below 20 °C); moderate temperature (location temperature between 20 and 45 °C); and high temperature (location temperature above 45 °C) [17].

As shown in Figure 4a, the number of studies completed in the three types of environments is not very different. Nevertheless, samples were preferably collected in high-temperature (6 studies) and moderate temperature environments (6 studies) instead of low-temperature environments (4 studies). Table 4 compiles all the data considered in the category of raw resources.

Table 4. Metagenomic studies reported in the last decade for water samples classified in the category of raw resources.

Sample Source	Source Properties		Metagenomic Approach	Vector/ Host	No. Positive Clones/ Total Clones	Enzyme	No. Citations *	Reference
	pH	T (°C)						
Surface seawater, South China Sea (Pacific Ocean)	-	-	Functional	BAC/ <i>E. coli</i>	6/-	β -glucosidase	95	[186]
Baltic Sea, Kołobrzeg, Poland (Atlantic Ocean)	-	0.8	Functional	Plasmid/ <i>E. coli</i>	1/1100	β -glucosidase	47	[187]
Lake Poraquê, Amazon (Brazil)	7.1	29.8	Sequence-based	-	-	β -glucosidase	6	[188]
Atlantis II brine pool, Red Sea (Indic Ocean)	5.3	68.0	Functional	Fosmid/ <i>E. coli</i>	5/10,656	Esterase	41	[189]
Surface seawater, South China Sea (Pacific Ocean)	-	-	Sequence-based	-	-	Esterase	25	[190]
Lobios hot spring, Ourense, Galicia (Spain)	>8.2	>76.0	Sequence-based and functional	Fosmid/ <i>E. coli</i>	6/11,600	Esterase	36	[191]
Urania, deep hypersaline anoxic basin interface, Mediterranean Sea (Atlantic Ocean)	-	14.0	Functional	Plasmid/ <i>E. coli</i>	41/90,800	Carboxylesterases	44	[50]
Surface seawater, South China Sea (Pacific Ocean)	-	-	Sequence-based	BAC/ <i>E. coli</i>	-/20,000	Laccase	93	[192]
Atlantis II brine pool, Red Sea (Indic Ocean)	5.4	68.1	Sequence-based	-	-	Mercuric reductase	30	[193]
Atlantis II brine pool, Red Sea (Indic Ocean)	5.7	68.4	Sequence-based	-	-	Thioredoxin reductase	4	[194]
Seawater, Gulf of Mexico (Atlantic Ocean)	-	-	Sequence-based	BAC/ <i>E. coli</i>	-	Ribulose 1,5-bisphosphate carboxylases/oxygenases	14	[195]
Seawater, long-Term Ecosystem Observatory site, New Jersey, USA (Atlantic Ocean)	-	-	Sequence-based	BAC/ <i>E. coli</i>	-	Fumarase	29	[196]
Surface seawater, South China Sea (Pacific Ocean)	8.2	15.0	Sequence-based	Plasmid/ <i>E. coli</i>	-/50,000	Fumarase	29	[196]
Atlantis II brine pool, Red Sea (Indic Ocean)	5.5	68.2	Sequence-based	-	-	Nitrilase	15	[197]
Atlantis II brine pool, Red Sea (Indic Ocean)	5.6	68.3	Sequence-based	-	-	3'-aminoglycoside phosphotransferase Beta-lactamase	11	[198]
Brackish water basin, Caspian Sea (Iran)	-	-	Sequence-based	-	-	L-asparaginases	0	[199]

* Obtained from the international database Scopus (21 March 2022).

3.1.1. Low-Temperature Environments

When thinking about low-temperature aquatic environments, thoughts are immediately associated with marine environments and/or high depth environments that are intrinsically related to high pressures. The decrease in temperature and the increase in depth cause a decreased diffusion of nutrients and energy and decreased abundance of prokaryotic cells, respectively. These extreme conditions require that microorganisms found in these zones have their adapted metabolism, for example, low concentrations of nutrients, which make them fascinating targets for the bioprospection of novel microbial capabilities and, accordingly, promising enzymes [200,201].

Low-temperature water samples were collected essentially in different geographical locations in the Atlantic Ocean. Surface seawater was collected at opposite ocean regions, particularly from an ecosystem observation site in New Jersey, USA [195], and the brackish Baltic Sea in Poland [187]. The latter recorded the lowest water temperature (0.8 °C), probably due to the fact that the Baltic sea was formed as a consequence of the last glaciation that occurred 10,000–15,000 years ago and, therefore, has undergone remarkable changes in its physicochemical characteristics [202]. In these different geographical locations, different groups of enzymes were reported, namely, a ribulose 1,5-bisphosphate carboxylase/oxygenase and a cytosolic β -glucosidase with a wide range of catalytic activities. Additionally, the chemocline of the Urania basin in the Mediterranean Sea was the subject of a metagenomic study to find interesting carboxylesterases, since it is a deep-sea anoxic hypersaline basin [50]. The extreme factors that characterize the Urania basin (hypersalinity, low temperature and anoxia) together with the typical features of chemoclines make this habitat accommodate a highly diverse microbial community with pronounced microbial activities, such as CO₂ fixation and exoenzyme activities [203].

3.1.2. Moderate Temperature Environments

In moderate temperature environments, samples of different types of water are included: groundwater/freshwater, hyper/saline water and brackish water.

Hyper/saline water samples from the surface of the South China Sea were the ones that most contributed to the metagenomic studies in this category. The seasonal average water temperature that falls into the range defined for this group (20–45 °C) and the unique environmental properties of the South China Sea potentially contribute to the diversity, novelty and uniqueness of genes encoding for valuable enzymes. Effectively, different groups of enzymes have already been explored in the South China Sea, including β -glucosidases, laccases and esterases [75,186,190,192].

As the lakes of the Amazon region remain unexplored, the freshwater metagenome of Lake Poraquê was functionally analysed. Being the largest hydrographic basin on Earth, the great genetic and metabolic diversity of microorganisms present in this important region may result in the discovery of new enzymes of biotechnological interest, such as enzymes involved in the degradation of plant cells walls [188].

The brackish samples of the Caspian Sea were also accessed since this environment presents a salinity and ionic concentration very similar to the human serum. In this way, there is a high probability that the secretory enzymes (more specifically, L-asparaginases) found in the Caspian Sea microbiome exhibit greater stability in the physiologic conditions of the human serum which can render them an interesting therapeutic applicability [199].

3.1.3. High-Temperature Environments

High-temperature habitats (>45 °C) are inhabited by heat-resistant microorganisms and some of these environments also combine other extreme conditions, for example, alkalinity, acidity, salinity, pressure and heavy metals [17]. Samples have been studied mainly from hyper/saline water environments and groundwater/freshwater environments.

Among the different aquatic environments, the hypersaline anoxic deep-sea basins in the Red Sea, namely, the Atlantis II deep brine pool, have received increased attention. These are characterized by a high temperature, extreme salinity, acidic pH, extremely low

levels of light and oxygen and high concentrations of heavy metals. In this way, this extreme environment is expected to be an attractive location for the search for biocatalysts that can function under harsh conditions, not just those that characterize the Red Sea. An esterase capable of acting in the presence of heavy metals; a mercuric reductase extremely relevant in the detoxification system for mercuric/organomercurial species; a nitrilase useful in bioremediation processes, fine chemicals and pharmaceuticals; a 3'-aminoglycoside phosphotransferase and a beta-lactamase with potential application as thermophilic selection markers; and a thioredoxin reductase important in the maintenance of the redox balance and counteracting oxidative stress inside cells are some examples of biocatalysts found in this extreme environment [189,193,194,197,198].

Another source commonly known for its high temperatures are the hot springs. A metagenomic library constructed from a groundwater sample from the Lobios hot spring in Spain was evaluated, by sequence-based and functional metagenomics approaches, given its high temperatures and alkaline pH values. Moreover, the microbial biodiversity and metagenomic potential of this source have not been sufficiently explored. This study reported a novel esterase belonging to family VIII and showed that the dominant prokaryotic phyla in this location, as in other hot springs on the planet, were *Deinococcus-Thermus*, *Proteobacteria*, *Firmicutes*, *Acidobacteria*, *Aquificae* and *Chloroflexi*. Additionally, the dominant archaeal phylum was *Thaumarchaeota* [191].

3.2. Human Manipulated Resources

Anthropogenic activities interfere negatively in many ways with the natural water cycle. Several water bodies, such as oceans, rivers and groundwater have been contaminated not only by natural events but particularly due to human interventions [204]. According to Figure 4a, 9 metagenomic studies were performed in contaminated water samples. This section was divided into groundwater/freshwater (4 studies) and coastal water (5 studies). Table 5 compiles all the data considered in the category of human manipulated resources.

Table 5. Metagenomic studies reported in the last decade for water samples classified in the category of human manipulated resources.

Sample Source	Source Properties		Metagenomic Approach	Vector/ Host	No. Positive Clones/ Total Clones	Enzyme	No. Citations *	Reference
	pH	T (°C)						
Contaminated river, East China University of Science and Technology, Shanghai (China)	-	-	Functional	Fosmid/ <i>E. coli</i>	6/20,400	Esterase	19	[205]
Coalbed water formation, Jharia coalfield, Jharkhand (India)	7.2	-	Functional	Fosmid/ <i>E. coli</i>	1/208	Phosphodiesterase	5	[206]
Messina harbour, Mediterranean Sea, Italy (Atlantic Ocean)		15.0		Plasmid/ <i>E. coli</i>	18/24,000			
Messina harbour (Int II), Mediterranean Sea, Italy (Atlantic Ocean)		15.0		Fosmid/ <i>E. coli</i>	208/5760			
Milazzo, Mediterranean Sea, Italy (Atlantic Ocean)	-	18.0	Functional	Plasmid/ <i>E. coli</i>	8/20,000	Carboxylesterases	44	[50]
Oil-contaminated coastal water, Kolguev Island, Barents Sea, Russia (Arctic Ocean)		3.0		Plasmid/ <i>E. coli</i>	34/142,000			
Oil-contaminated Murmansk Port, Barents Sea, Russia (Arctic Ocean)		5.0		Plasmid/ <i>E. coli</i>	43/108,000			
Eryuan Niujie hot spring, Yunnan (China)	7.0	58.0	Functional	BAC/ <i>E. coli</i>	10/68,352	Lipase	11	[207]
Oil industry products contaminated groundwater (Czech Republic)	6.9	8.5	Sequence-based	-	-	Haloalkane dehalogenases	3	[208]

* Obtained from the international database Scopus (21 March 2022).

3.2.1. Groundwater/Freshwater

The main anthropogenic sources of water contamination are refineries, mines, factories and wastewater treatment plants, among others [204].

Over the past 10 years, different metagenomic studies have been executed in contaminated groundwater and freshwater sources for the acquisition of novel enzymes. Some examples of contaminated sources are the formation of water in a coalbed in Jharia coalfield (India) which is defined as an extreme environment [206], Eryuan Niujie hot spring in Yunnan (China), which has a high content of fats due to the wastes resulting from the livestock slaughter that occurs in the vicinity of the hot spring [207], and groundwater from an area in the Czech Republic that has been incessantly contaminated with various products from an oil industry for over 50 to 70 years [208].

The activities associated with each of the sites employ a certain continuous selective pressure on the microorganisms that live in these environments to develop enzymes capable of acting in the production of biodiesel, degradation of organophosphorus compounds and halogenated pollutants, respectively [206–208].

3.2.2. Coastal Water

The category of coastal waters is essentially composed of water samples from oil-contaminated harbours due to the numerous ships circulating in the waters of these areas each year and also due to the unintentional spills of hydrocarbons that may occur during the loading and unloading of petroleum-derived substances. The main pollutant responsible for the contamination of these sites is oil and the studies have mainly been focused on the Mediterranean Sea in Italy and the Barents Sea in Russia, allowing the finding of robust carboxylesterases [50].

The importance of the metagenomic analysis of water samples of these types of sources is justified by the abundance of microbial species capable of degrading hydrocarbons which can be potentially applied in the bioremediation of ecosystems. To this end, additional enrichments with crude oil or specific hydrocarbons (e.g., pyrene, naphthalene and phenanthrene) are carried out to mimic the place from which they are isolated.

3.3. Unspecified Resource

The tropical underground water of the Yucatán Aquifer in Mexico was the only resource considered unspecified. Nevertheless, it is a very interesting resource of freshwater since it consists of very permeable and porous limestone that allows the infiltration of water into the deepest layers of the soil. Additionally, the Yucatán Aquifer presents cracks or interconnected spaces that allow water from distant zones and sources to move freely, carrying a collection of microorganisms from different places. Thus, although it should be a natural selection process that favours some microorganisms by eliminating others, it may also present a high microbial diversity from diverse origins [209].

In this way, this aquifer can represent a potential and interesting source for the acquisition of a catalogue of enzymes suitable for the degradation of natural polymers, including proteins.

4. Soil versus Water

As previously demonstrated, soil and water have both been reported as promising sources of biocatalysts. Nevertheless, the number of metagenomic studies focused on soil have been considerably superior, as illustrated in Figure 5. Between 2010 and 2021, the period considered in this review, the soil was always a preferred source to search for new biocatalysts, representing more than 69% of the studies each year. Additionally, it is also important to highlight that the number of metagenomic studies for the identification of enzymes has been decreasing for both natural resources. In fact, since 2017, a significant reduction in the number of studies was observed but in 2021 this number went up again (Figure 5), probably as a consequence of the pandemic situation which, in general, resulted in a sharp increase in publication volume [210]. In the last years, we have witnessed a

change in the focus of metagenomic studies from functionality to taxonomy and gene annotation. The development of more accurate software allows access to the complex data generated by the sequence-based metagenomics approach. Consequently, it is possible to explore the biosynthetic gene cluster diversity and to understand the significant role of the microorganisms in the global biogeochemical cycles.

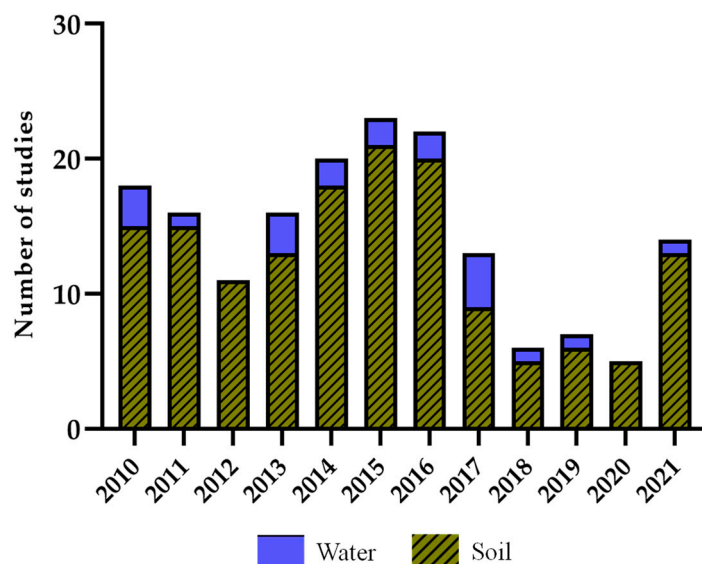


Figure 5. Number of metagenomic studies performed between 2010 and 2021 using water and soil samples to find promising enzymes.

The global distribution of the several metagenomic studies performed with soil and water samples is illustrated in Figure 6a. This figure shows that soil and/or water samples from five continents (America, Europe, Asia, Africa and Antarctica) and four oceans (Atlantic, Pacific, Arctic and Indic) were already explored through metagenomic studies aiming at the discovery of new enzymes. Regarding the soil samples, Asia was the continent with the higher number of studies (~52%), followed by Europe (~24%) and America (~13%) (Figure 6b). The samples were mostly collected from human manipulated environments, which can be justified by the higher demographic density and industrial activity attributed to these continents. The number of studies performed with soil samples from Africa or Antarctica was very low (<2%). When considering the studies performed in marine sediments, a higher percentage was found for the Pacific Ocean (~5%) (Figure 6b).

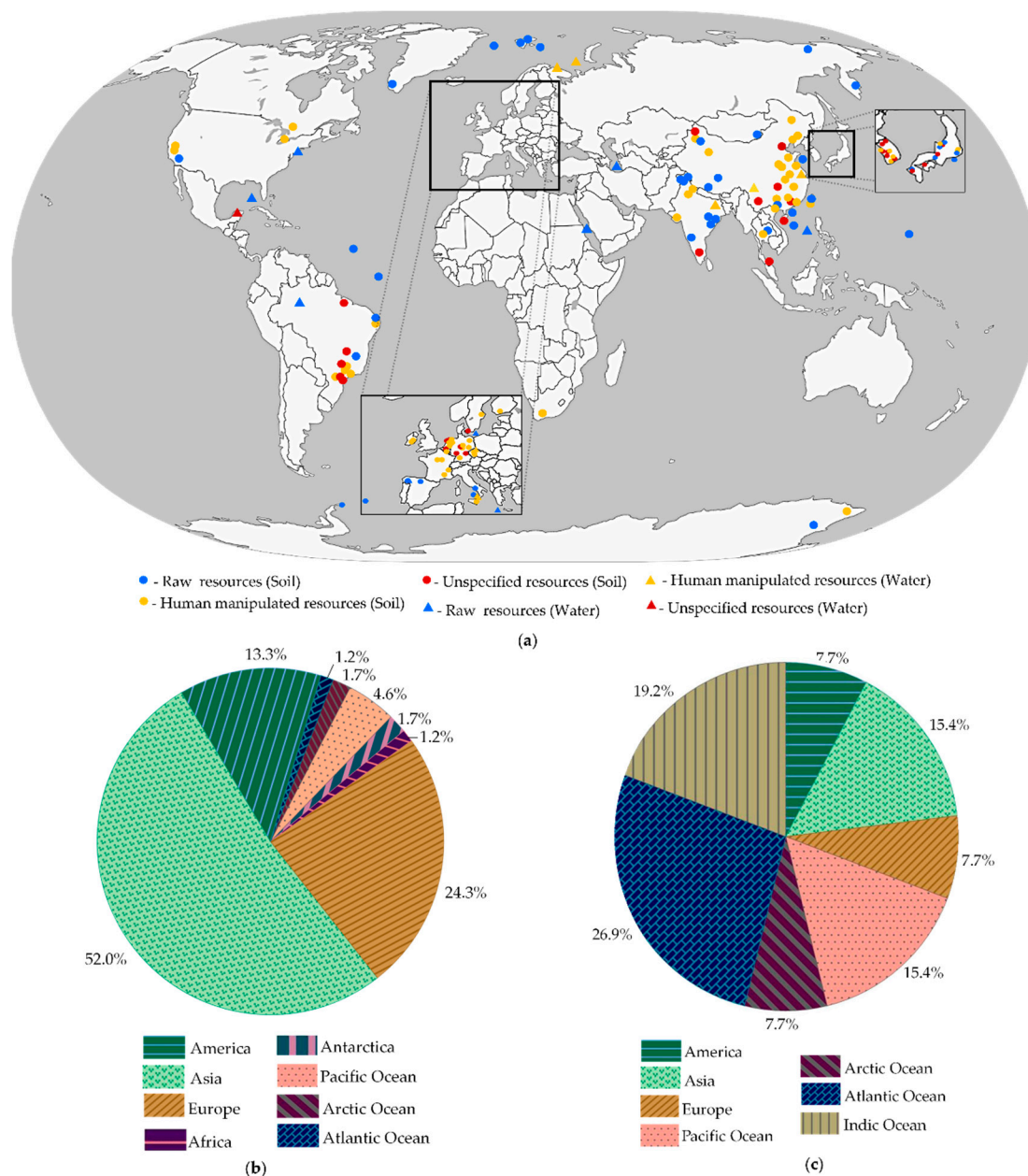


Figure 6. Distribution of the several metagenomic studies performed with soil and water samples in terms of: (a) global representation; (b) percentage of soil and sediment samples collected in each continent and ocean; (c) percentage of water samples collected in each continent and ocean.

On the other hand, the marine water samples studied by metagenomic approaches were mostly collected in the Atlantic Ocean (~27%), Indic Ocean (~19%) and Pacific Ocean (~15%) (Figure 6c). In terms of continental water, samples were obtained from Asia (~15%), Europe (~8%) and America (~8%).

The main groups of enzymes identified in the metagenomic studies include lipolytic enzymes, glycosyl hydrolases, oxidoreductases, phosphatases/phytases and proteases. The global distribution of these enzymes according to the type of sample used in the metagenomic study is represented in Figure 7a. The soil samples from Asia, Europe and America generally resulted in the identification of lipolytic enzymes and glycosyl hydrolases (Figure 7b). Oxidoreductases were mostly obtained from Asian soil samples. On the other hand, some phosphatases/phytases were identified in samples from European soils. Additionally, the metagenomic studies performed with both European and Asian soil

samples showed the presence of other types of enzymes such as RNase [69], rhodanase [70], trehalose synthase [143], sulfatases [145] or wax ester synthase [142]. The soil samples collected in Africa and Antarctica, as well as the marine sediments from the Pacific, Antarctic and Atlantic Oceans, essentially allowed the identification of lipolytic enzymes.

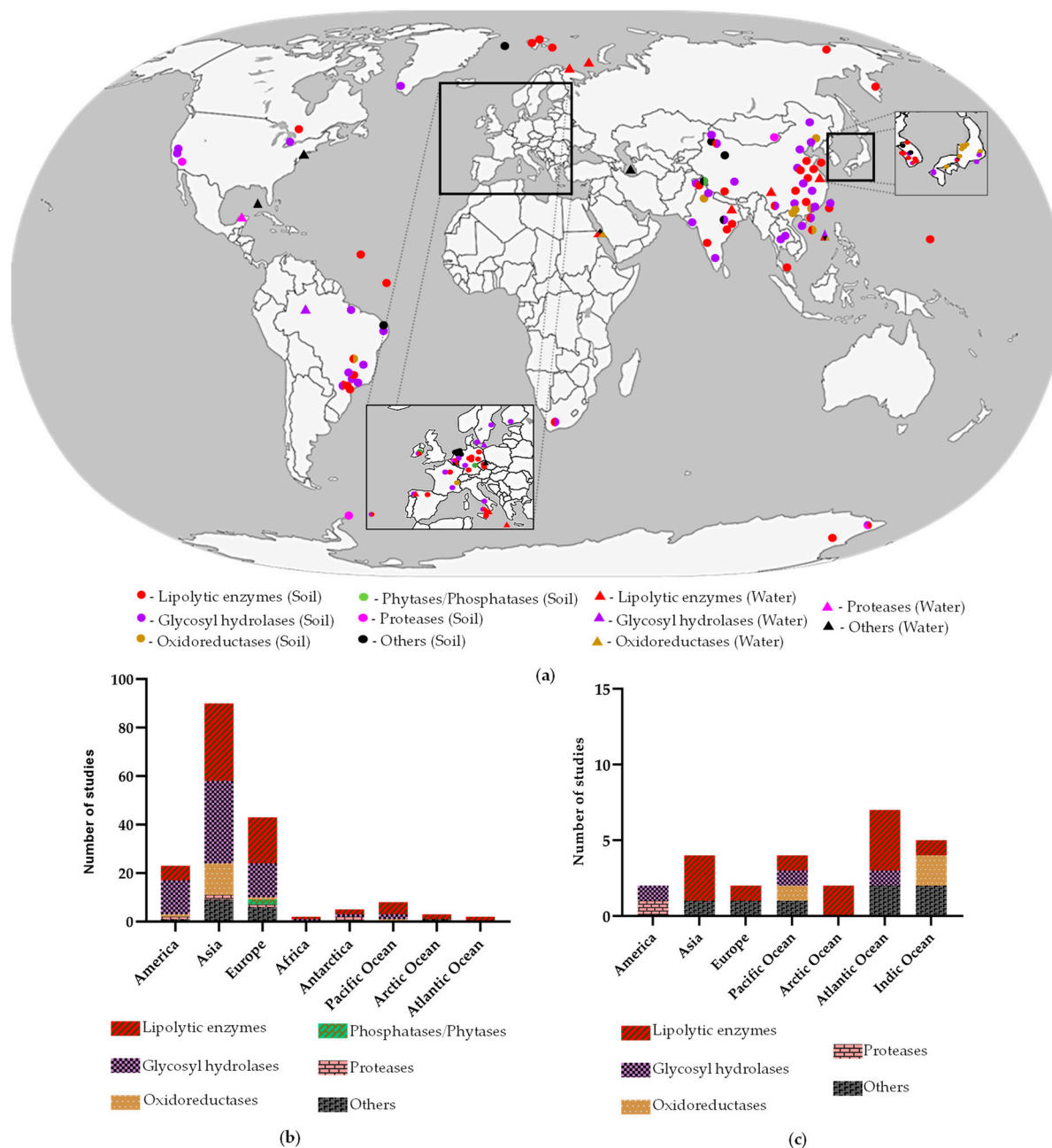


Figure 7. Distribution of the main groups of enzymes found in the metagenomic studies performed with soil and water samples in terms of: (a) global representation; (b) main groups of enzymes (%) identified in soil and sediment samples collected in each continent and ocean; (c) main group of enzymes (%) identified in water samples collected in each continent and ocean.

For the water samples, the lipolytic enzymes were also the predominant group, being identified in almost all the studied locations (except for samples from America) (Figure 7c). Nevertheless, proteases were only identified in samples collected in America. Glycosyl hydrolases were found in continental water from America and marine water from the Pacific and Atlantic Oceans. On the other hand, oxidoreductases were found in marine water

samples from the Pacific and Indic Oceans. Similar to the soil, other enzymatic activities were identified in the water samples (except for samples from America and the Arctic Ocean) such as nitrilase [197], beta-lactamase [198], L-asparaginases [199], fumarase [196] or haloalkane dehalogenases [208]. However, no phosphatases/phytases were identified in the metagenomic studies of water.

Overall, it was discovered that lipolytic enzymes, glycosyl hydrolases, oxidoreductases and proteases could be found in both natural resources (Figure 8). Nevertheless, phosphatases and phytases were only identified in soil samples. Furthermore, the enzymatic activities included in the “others” group were significantly different for soil and water samples (Tables 1–5).

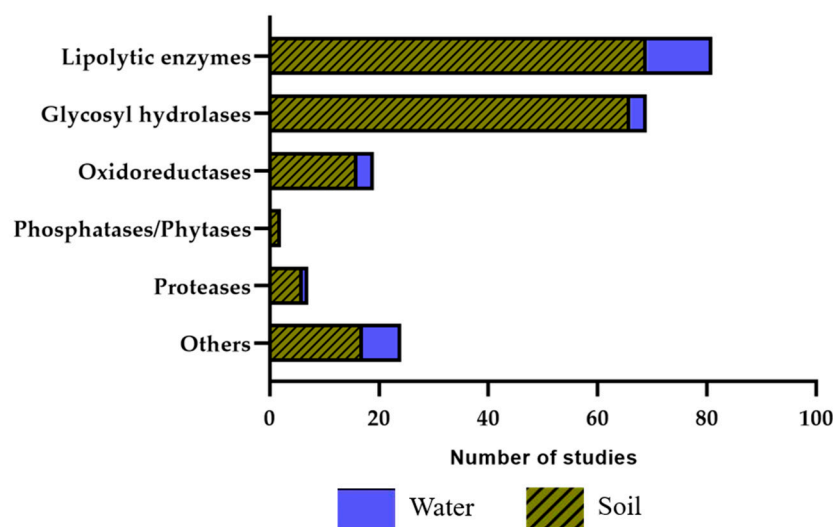


Figure 8. Number of metagenomic studies reporting the presence of lipolytic enzymes, glycosyl hydrolases, oxidoreductases, phosphatases/phytases, proteases and other types of enzymes in soil and water samples.

The five groups of enzymes mostly identified in the metagenomic studies (namely, lipolytic enzymes, glycosyl hydrolases, oxidoreductases, phosphatases/phytases and proteases) correspond to catalytic activities with a great interest in the industry. Lipolytic enzymes, such as lipases and esterases, can catalyse either the hydrolysis or the synthesis of ester bonds. They are considered robust enzymes, which can resist the harsh conditions of some industrial processes such as high temperature and pH, and the presence of organic solvents. These enzymes have been applied in the food, cosmetic, pharmaceutical, detergent, laundry and oleo-chemical industries [211]. Lipases and esterases showing resistance to high pH values (>8.5) were mostly found in soil samples, namely, petroleum hydrocarbons-contaminated soil (esterase, optimal pH = 9.0 [117]), Brazilian cerrado soil (lipase, optimal pH = 9.0–9.5 [165]), compost units (esterase, optimal pH = 10.0 [114]), fat-contaminated soil (lipase, optimal pH = 10.0 [129]) or cold desert soil (esterase, optimal pH = 11.0 [39]). On the other hand, lipases and esterases with tolerance to high temperatures (>65 °C) were identified in hot spring soils (esterase, optimal temperature 80 °C [43]; lipase, optimal temperature 65 °C [61]) and compost units (esterase, optimal temperature 75 °C [43,121]). Additionally, in water samples from the Red Sea (esterase, optimal temperature 65 °C [189]) and the South China Sea (esterase, optimal temperature 65 °C [190]), thermo and halo-tolerant esterases were found. Furthermore, other interesting enzymes able to catalyse the hydrolysis of ester compounds were obtained from compost (cutinase [59]), wheat field soil (feruloyl esterase [116]) or contaminated soil from wood-processing industries (carboxylesterases [50]).

The group of glycosyl hydrolases includes several enzymes which promote the breakdown of carbohydrates into simple sugars through the hydrolysis of specific glycosidic

bonds. This kind of enzyme has been widely explored in biorefining processes for the hydrolysis of different polysaccharides from plant origin and the subsequent production of important added-value products like biofuels. Furthermore, glycosyl hydrolases are also interesting enzymes for the cosmetic (e.g., toothpaste additives), food (e.g., dairy, baking or brewing processes) and feed industries [212]. Three metagenomic studies performed with water samples from marine water and freshwater led to the identification of β -glucosidases [186–188]—important enzymes in the hydrolysis of short-chain oligosaccharides into glucose. As expected, enzymes belonging to the family of cellulases (e.g., endoglucanases and β -glucosidases), xylanases (e.g., endoxylanases and β -xylosidase) and amylases were widely found in soil samples. Chitinases able to catalyse the hydrolysis of chitin commonly present in the exoskeleton of some animals were also identified in metagenomic studies with soil. The natural presence of plant and animal detritus in soils justifies the existence of microorganisms with the catalytic ability for the conversion of cellulose, xylan, starch or chitin.

The family of proteases include the enzymes which promote proteolysis, i.e., the breakdown of proteins into smaller peptides or amino acids. Proteases are well-established enzymes in the food and feed industry, being used as stabilizers, meat tenderizers and additives for better digestion, or even for the improvement of brewing and baking processes. They can also be applied in leather and detergent industries, as well as in photography or therapeutic uses [213]. According to the metagenomic studies analysed here, proteolytic activity seems to be more common in soils than in water. Alkaline proteases were found in desert environments [66], forest soil [177], hot springs sediments [67] and oil-contaminated soil [111].

Oxidoreductases are a vast number of enzymes able to catalyse the transfer of electrons from one molecule (reductant) to another (oxidant). This kind of enzymes is regarded as important biocatalysts in the textile (dye bleaching), pulp and paper (bleaching), food and beverage (stabilizer) and pharmaceutical industries (synthesis of bioactive compounds), as well as in biorefining processes for the bioconversion of lignocellulose [214]. They can also have an important role in the bioremediation of industrial and municipal wastewater contaminated with organic compounds such as textile dyes, pharmaceuticals, hormones or personal care products [215]. Diverse oxidoreductases were reported in the metagenomic studies analysed here, including laccases [154,192], oxygenases [137], alcohol and aldehyde dehydrogenases [63,65], D-amino acid oxidases [136] and bilirubin oxidases [135]. Nevertheless, the majority of these enzymes were identified in soil samples.

Phosphatases are enzymes involved in the cleavage of ester bonds and the release of phosphate groups. Phytases are a type of phosphatases that act in the hydrolysis of phytic acid, a specific organic form of phosphorous. These enzymes are of great interest in human and animal nutrition by reducing the phytate content of food products and contributing to more efficient digestion and absorption of phosphorus [216]. Phytases are common and very useful biocatalysts in soils since they are considered as primary agents for dephosphorylation [217]. In fact, phytases were only found in the metagenomic studies performed with soil samples [141,159].

5. General Conclusions and Future Perspectives

Metagenomics proved to be an efficient technique to explore natural resources such as soil and water. Following either a sequence- or a function-based approach, several enzymes with distinct catalytic activities were identified. In the last decade, the number of metagenomic studies performed with soil samples was considerably higher than compared with water samples. This fact can be related to some limitations reported for DNA extraction. When working with water, high volumes of samples need to be collected and filtered to allow the extraction of an appropriate quantity of DNA considered representative of the microbial communities present in the environment. Furthermore, water sources are immensely vast and, in most cases, not fully attainable without specific equipment and protection (e.g., oceans, seas and groundwater). On the other hand, as terrestrial

living organisms, we found the soil as a much more accessible and near environment. Nevertheless, it is clear that the biotechnological potential of soil remains barely explored. The global distribution of the metagenomic studies carried out for enzyme discovery in the last decade noticeably shows that a vast number of promising environments are still waiting for their potential to be unravelled. The lack of economic and human resources in the African continent may justify the reporting of only two metagenomic studies, namely with compost samples from the same composting facility. However, other continents like America and Europe could also be explored more. The same conclusions are obviously taken for water samples.

The catalytic activities found in the metagenomic studies were mostly distributed in five representative groups. The origin and composition of the samples are generally connected with the biocatalytic potential of the microbial communities. Parameters like the pH and temperature of the sampling location are frequently related to the optimal conditions reported for the biocatalysts. Additionally, in most cases, it was possible to establish an association between the type of enzymes and the main constituents of the sample. Lipolytic enzymes were often found in environments containing petroleum hydrocarbons, oils or fat. Glycosyl hydrolases were mostly identified in soils since these environments are naturally composed of plant and animal debris. Thus, the microbial communities take advantage of producing these types of enzymes to convert the complex polysaccharides of plant and animal origin. Similarly, phytases are enzymes with a catalytic action that properly fits the soil environments where phosphorus assumes an important role. The presence of oxidoreductases can also be more expected in soils due to the key role they have in the conversion of lignin from plants. For proteases, no clear association was established for the metagenomic data reported.

In the last years, more accurate and advanced software to access and analyse the complex sequencing data generated in metagenomic studies have been developed. This fact greatly contributed to a better understanding of the interactions established between microbial communities and the environment by accessing important information about biosynthetic routes and taxonomic annotation. As a consequence, the motivation of the metagenomic studies slightly diverged. The studies initially focused on microbial functionality have been replaced by studies exclusively directed to whole-genome sequencing and metabolite prediction. It is expected that metagenomics consolidates in the future its status as a key technique to unravel the biotechnological potential of microbial resources, thus contributing to the search for novel and effective bioactive compounds according to the market trends.

Author Contributions: Conceptualization, J.S., S.C.S. and L.R.R.; bibliographic survey, J.S. and A.M.A.C.; writing—original draft preparation, J.S. and S.C.S.; writing—review and editing A.M.A.C. and L.R.R.; project administration, S.C.S. and L.R.R.; funding acquisition S.C.S. and L.R.R. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit, the Project LIGNOZYMES—Metagenomics approach to unravel the potential of lignocellulosic residues towards the discovery of novel enzymes (POCI-01-0145-FEDER-029773) and the Project B3iS—Biodiversity and Bioprospecting of Biosurfactants in Saline Environments (PTDC/BII-BIO/5554/2020).

Data Availability Statement: Not applicable.

Acknowledgments: J.S. and A.M.A.C. acknowledge their research grants UMINHO/BIM/2020/28 and UMINHO/BPD/37/2018, respectively, under the scope of the project LIGNOZYMES (POCI-01-0145-FEDER-029773).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Arocena, J.M.; Driscoll, K.G. Natural resources of the world. In *Knowledge for Sustainable Development: An Insight into the Encyclopaedia of Life Support Systems*; UNESCO/EOLSS: Zurich, Switzerland, 2002; pp. 261–290.

2. Kattumuri, R. Sustaining natural resources in a changing environment: Evidence, policy and impact. *Contemp. Soc. Sci.* **2018**, *13*, 1–16. [[CrossRef](#)]
3. Lampert, A. Over-exploitation of natural resources is followed by inevitable declines in economic growth and discount rate. *Nat. Commun.* **2019**, *10*, 1419. [[CrossRef](#)] [[PubMed](#)]
4. Kirsch, S. Running out? Rethinking resource depletion. *Extr. Ind. Soc.* **2020**, *7*, 838–840. [[CrossRef](#)] [[PubMed](#)]
5. Chettri, D.; Verma, A.K.; Verma, A.K. Innovations in CAZyme gene diversity and its modification for biorefinery applications. *Biotechnol. Rep.* **2020**, *28*, e00525. [[CrossRef](#)] [[PubMed](#)]
6. Gudiña, E.J.; Amorim, C.; Braga, A.; Costa, Â.; Rodrigues, J.L.; Silvério, S.; Rodrigues, L.R. Biotech green approaches to unravel the potential of residues into valuable products. In *Sustainable Green Chemical Processes and Their Allied Applications*; Inamuddin, M., Asiri, A.M., Eds.; Springer: Cham, Switzerland, 2020; pp. 97–150.
7. Berini, F.; Casciello, C.; Marcone, G.L.; Marinelli, F. Metagenomics: Novel enzymes from non-culturable microbes. *FEMS Microbiol. Lett.* **2017**, *364*, fnx211. [[CrossRef](#)] [[PubMed](#)]
8. Costa, Â.M.A.; Santos, A.O.; Sousa, J.; Rodrigues, J.L.; Gudiña, E.J.; Silvério, S.C.; Rodrigues, L.R. Improved method for the extraction of high-quality DNA from lignocellulosic compost samples for metagenomic studies. *Appl. Microbiol. Biotechnol.* **2021**, *105*, 8881–8893. [[CrossRef](#)] [[PubMed](#)]
9. Robinson, S.L.; Piel, J.; Sunagawa, S. A roadmap for metagenomic enzyme discovery. *Nat. Prod. Rep.* **2021**, *38*, 1994–2023. [[CrossRef](#)] [[PubMed](#)]
10. Batista-García, R.A.; del Rayo Sánchez-Carbente, M.; Talia, P.; Jackson, S.A.; O’Leary, N.D.; Dobson, A.D.W.; Folch-Mallol, J.L. From lignocellulosic metagenomes to lignocellulolytic genes: Trends, challenges and future prospects. *Biofuels Bioprod. Biorefining* **2016**, *10*, 864–882. [[CrossRef](#)]
11. Wang, H.; Hart, D.J.; An, Y. Functional metagenomic technologies for the discovery of novel enzymes for biomass degradation and biofuel production. *BioEnergy Res.* **2019**, *12*, 457–470. [[CrossRef](#)]
12. Escuder-Rodríguez, J.-J.; DeCastro, M.-E.; Becerra, M.; Rodríguez-Belmonte, E.; González-Siso, M.-I. Advances of functional metagenomics in harnessing thermozymes. In *Metagenomics: Perspectives, Methods, and Applications*; Academic Press: Cambridge, MA, USA, 2018; pp. 289–307.
13. Koorevaar, G.P.; Menelik, C.D. (Eds.) 1 Composition and physical properties of soils. In *Developments in Soil Science*; Elsevier: Amsterdam, The Netherlands, 1983; pp. 1–36.
14. Bhattacharyya, T.; Pal, K.D. The Soil: A natural resource. In *Soil Science: An Introduction*; Rattan, R.K., Katyal, J.C., Dwivedi, B.S., Sarkar, A.K., Tapas Bhattacharyya, J.C., Tarafdar, S.K., Eds.; ISSS: New Delhi, India, 2015; pp. 1–19.
15. Jindal, S. Microbes in soil and their Metagenomics. In *Microbial Diversity, Interventions and Scope*; Sharma, S.G., Neeta Raj Sharma, M.S., Eds.; Springer: Singapore, 2020; pp. 85–96.
16. Wang, L.; D’Odorico, P. Decomposition and mineralization. In *Reference Module in Earth Systems and Environmental Sciences*; Elsevier: Amsterdam, The Netherlands, 2013; pp. 280–285.
17. Merino, N.; Aronson, H.S.; Bojanova, D.P.; Feyhl-Buska, J.; Wong, M.L.; Zhang, S.; Giovannelli, D. Living at the extremes: Extremophiles and the limits of life in a planetary context. *Front. Microbiol.* **2019**, *10*, 780. [[CrossRef](#)] [[PubMed](#)]
18. Mirete, S.; Morgante, V.; González-Pastor, J.E. Functional metagenomics of extreme environments. *Curr. Opin. Biotechnol.* **2016**, *38*, 143–149. [[CrossRef](#)] [[PubMed](#)]
19. Sharma, S.; Khan, F.G.; Qazi, G.N. Molecular cloning and characterization of amylase from soil metagenomic library derived from Northwestern Himalayas. *Appl. Microbiol. Biotechnol.* **2010**, *86*, 1821–1828. [[CrossRef](#)] [[PubMed](#)]
20. Liu, Y.; Lei, Y.; Zhang, X.; Gao, Y.; Xiao, Y.; Peng, H. Identification and phylogenetic characterization of a new subfamily of α -amylase enzymes from marine microorganisms. *Mar. Biotechnol.* **2012**, *14*, 253–260. [[CrossRef](#)] [[PubMed](#)]
21. Vester, J.K.; Glaring, M.A.; Stougaard, P. Discovery of novel enzymes with industrial potential from a cold and alkaline environment by a combination of functional metagenomics and culturing. *Microb. Cell Fact.* **2014**, *13*, 72. [[CrossRef](#)] [[PubMed](#)]
22. Gupta, R.; Govil, T.; Capalash, N.; Sharma, P. Characterization of a glycoside hydrolase family 1 β -galactosidase from hot spring metagenome with transglycosylation activity. *Appl. Biochem. Biotechnol.* **2012**, *168*, 1681–1693. [[CrossRef](#)] [[PubMed](#)]
23. Zhang, X.; Li, H.; Li, C.J.; Ma, T.; Li, G.; Liu, Y.H. Metagenomic approach for the isolation of a thermostable β -galactosidase with high tolerance of galactose and glucose from soil samples of Turpan Basin. *BMC Microbiol.* **2013**, *13*, 237. [[CrossRef](#)] [[PubMed](#)]
24. Okano, H.; Ozaki, M.; Kanaya, E.; Kim, J.J.; Angkawidjaja, C.; Koga, Y.; Kanaya, S. Structure and stability of metagenome-derived glycoside hydrolase family 12 cellulase (LC-CelA) a homolog of Cel12A from *Rhodothermus marinus*. *FEBS Open Bio* **2014**, *4*, 936–946. [[CrossRef](#)] [[PubMed](#)]
25. Sae-Lee, R.; Boonmee, A. Newly derived GH43 gene from compost metagenome showing dual xylanase and cellulase activities. *Folia Microbiol.* **2014**, *59*, 409–417. [[CrossRef](#)] [[PubMed](#)]
26. Yeh, Y.-F.; Chang, S.C.-Y.; Kuo, H.-W.; Tong, C.-G.; Yu, S.-M.; Ho, T.-H.D. A metagenomic approach for the identification and cloning of an endoglucanase from rice straw compost. *Gene* **2013**, *519*, 360–366. [[CrossRef](#)]
27. Suleiman, M.; Schröder, C.; Klippel, B.; Schäfers, C.; Krüger, A.; Antranikian, G. Extremely thermoactive archaeal endoglucanase from a shallow marine hydrothermal vent from Vulcano Island. *Appl. Microbiol. Biotechnol.* **2019**, *103*, 1267–1274. [[CrossRef](#)] [[PubMed](#)]
28. Schröder, C.; Elleuche, S.; Blank, S.; Antranikian, G. Characterization of a heat-active archaeal β -glucosidase from a hydrothermal spring metagenome. *Enzym. Microb. Technol.* **2014**, *57*, 48–54. [[CrossRef](#)]

29. Cao, L.C.; Wang, Z.J.; Ren, G.H.; Kong, W.; Li, L.; Xie, W.; Liu, Y.H. Engineering a novel glucose-tolerant β -glucosidase as supplementation to enhance the hydrolysis of sugarcane bagasse at high glucose concentration. *Biotechnol. Biofuels* **2015**, *8*, 202. [[CrossRef](#)] [[PubMed](#)]
30. Matsuzawa, T.; Yaoi, K. Screening, identification, and characterization of a novel saccharide-stimulated β -glycosidase from a soil metagenomic library. *Appl. Microbiol. Biotechnol.* **2017**, *101*, 633–646. [[CrossRef](#)] [[PubMed](#)]
31. Klippel, B.; Sahn, K.; Basner, A.; Wiebusch, S.; John, P.; Lorenz, U.; Peters, A.; Abe, F.; Takahashi, K.; Kaiser, O.; et al. Carbohydrate-active enzymes identified by metagenomic analysis of deep-sea sediment bacteria. *Extremophiles* **2014**, *18*, 853–863. [[CrossRef](#)] [[PubMed](#)]
32. Kaushal, G.; Kumar, J.; Sangwan, R.S.; Singh, S.P. Metagenomic analysis of geothermal water reservoir sites exploring carbohydrate-related thermozymes. *Int. J. Biol. Macromol.* **2018**, *119*, 882–895. [[CrossRef](#)] [[PubMed](#)]
33. Colombo, L.T.; de Oliveira, M.N.V.; Carneiro, D.G.; de Souza, R.A.; Alvim, M.C.T.; dos Santos, J.C.; da Silva, C.C.; Vidigal, P.M.P.; da Silveira, W.B.; Passos, F.M.L. Applying functional metagenomics to search for novel lignocellulosic enzymes in a microbial consortium derived from a thermophilic composting phase of sugarcane bagasse and cow manure. *Antonie Leeuwenhoek* **2016**, *109*, 1217–1233. [[CrossRef](#)] [[PubMed](#)]
34. Strazzulli, A.; Cobucci-Ponzano, B.; Iacono, R.; Giglio, R.; Maurelli, L.; Curci, N.; Schiano-di-Cola, C.; Santangelo, A.; Contursi, P.; Lombard, V.; et al. Discovery of hyperstable carbohydrate-active enzymes through metagenomics of extreme environments. *FEBS J.* **2020**, *287*, 1116–1137. [[CrossRef](#)] [[PubMed](#)]
35. Verma, D.; Kawarabayasi, Y.; Miyazaki, K.; Satyanarayana, T. Cloning, expression and characteristics of a novel alkalistable and thermostable xylanase encoding gene (Mxyl) retrieved from compost-soil metagenome. *PLoS ONE* **2013**, *8*, e52459. [[CrossRef](#)]
36. Knapik, K.; Becerra, M.; González-Siso, M.I. Microbial diversity analysis and screening for novel xylanase enzymes from the sediment of the Lobios Hot Spring in Spain. *Sci. Rep.* **2019**, *9*, 11195. [[CrossRef](#)] [[PubMed](#)]
37. Matsuzawa, T.; Kimura, N.; Suenaga, H.; Yaoi, K. Screening, identification, and characterization of α -xylosidase from a soil metagenome. *J. Biosci. Bioeng.* **2016**, *122*, 393–399. [[CrossRef](#)] [[PubMed](#)]
38. Dai, Y.; Yang, F.; Liu, X.; Wang, H. The discovery and characterization of a novel chitinase with dual catalytic domains from a Qinghai-Tibetan Plateau wetland soil metagenome. *Int. J. Biol. Macromol.* **2021**, *188*, 482–490. [[CrossRef](#)] [[PubMed](#)]
39. Hu, X.P.; Heath, C.; Taylor, M.P.; Tuffin, M.; Cowan, D. A novel, extremely alkaliphilic and cold-active esterase from Antarctic desert soil. *Extremophiles* **2012**, *16*, 79–86. [[CrossRef](#)] [[PubMed](#)]
40. Martínez-martínez, M.; Alcaide, M.; Tchigvintsev, A.; Reva, O.; Polaina, J.; Bargiela, R.; Guazzaroni, M.-E.; Chicote, Á.; Canet, A.; Valero, F.; et al. Biochemical diversity of carboxyl esterases and lipases from Lake Arreo (Spain): A metagenomic approach. *Appl. Environ. Microbiol.* **2013**, *79*, 3553–3562. [[CrossRef](#)] [[PubMed](#)]
41. Sudan, A.K.; Vakhlu, J. Isolation of a thioesterase gene from the metagenome of a mountain peak, Apharwat, in the northwestern Himalayas. *3 Biotech* **2013**, *3*, 19–27. [[CrossRef](#)]
42. Okano, H.; Hong, X.; Kanaya, E.; Angkawidjaja, C.; Kanaya, S. Structural and biochemical characterization of a metagenome-derived esterase with a long N-terminal extension. *Protein Sci.* **2015**, *24*, 93–104. [[CrossRef](#)]
43. Leis, B.; Angelov, A.; Mientus, M.; Li, H.; Pham, V.T.T.; Lauinger, B.; Bongen, P.; Pietruszka, J.; Gonçalves, L.G.; Sutherland, R. Identification of novel esterase-active enzymes from hot environments by use of the host bacterium *Thermus thermophilus*. *Front. Microbiol.* **2015**, *6*, 275. [[CrossRef](#)] [[PubMed](#)]
44. Sudan, A.K.; Vakhlu, J. Isolation and in silico characterization of novel esterase gene with β -lactamase fold isolated from metagenome of north western Himalayas. *3 Biotech* **2015**, *5*, 553–559. [[CrossRef](#)]
45. De Santi, C.; Ambrosino, L.; Tedesco, P.; de Pascale, D.; Zhai, L.; Zhou, C.; Xue, Y.; Ma, Y. Identification and characterization of a novel salt-tolerant esterase from a Tibetan glacier metagenomic library. *Biotechnol. Prog.* **2015**, *31*, 890–899. [[CrossRef](#)]
46. Zarafeta, D.; Moschidi, D.; Ladoukakis, E.; Gavrilov, S.; Chrysina, E.D.; Chatziioannou, A.; Kublanov, I.; Skretas, G.; Kolisis, F.N. Metagenomic mining for thermostable esterolytic enzymes uncovers a new family of bacterial esterases. *Sci. Rep.* **2016**, *6*, 38886. [[CrossRef](#)]
47. Petrovskaya, L.E.; Novototskaya-Vlasova, K.A.; Spirina, E.V.; Durdenko, E.V.; Lomakina, G.Y.; Zavialova, M.G.; Nikolaev, E.N.; Rivkina, E.M. Expression and characterization of a new esterase with GCSAG motif from a permafrost metagenomic library. *FEMS Microbiol. Ecol.* **2016**, *92*, fiw046. [[CrossRef](#)] [[PubMed](#)]
48. De Santi, C.; Altermark, B.; Pierechod, M.M.; Ambrosino, L.; De Pascale, D.; Willassen, N.P. Characterization of a cold-active and salt tolerant esterase identified by functional screening of Arctic metagenomic libraries. *BMC Biochem.* **2016**, *17*, 1–13. [[CrossRef](#)] [[PubMed](#)]
49. Gao, W.; Wu, K.; Chen, L.; Fan, H.; Zhao, Z.; Gao, B.; Wang, H.; Wei, D. A novel esterase from a marine mud metagenomic library for biocatalytic synthesis of short-chain flavor esters Wenyuan. *Microb. Cell Fact.* **2016**, *15*, 41. [[CrossRef](#)] [[PubMed](#)]
50. Popovic, A.; Hai, T.; Tchigvintsev, A.; Hajighasemi, M.; Nocek, B.; Khusnutdinova, A.N.; Brown, G.; Glinos, J.; Flick, R.; Skarina, T.; et al. Activity screening of environmental metagenomic libraries reveals novel carboxylesterase families. *Sci. Rep.* **2017**, *7*, 44103. [[CrossRef](#)] [[PubMed](#)]
51. Li, P.-Y.; Yao, Q.-Q.; Wang, P.; Zhang, Y.; Li, Y.; Zhang, Y.-Q.; Hao, J.; Zhou, B.-C.; Chen, X.-L.; Shi, M.; et al. A novel subfamily esterase with a homoserine transacetylase-like fold but no transferase activity. *Appl. Environ. Microbiol.* **2017**, *83*, e00131-17. [[CrossRef](#)] [[PubMed](#)]

52. Jayanath, G.; Mohandas, S.P.; Kachiprath, B.; Solomon, S.; Sajeevan, T.P.; Bright Singh, I.S.; Philip, R. A novel solvent tolerant esterase of GDSGG motif subfamily from solar saltern through metagenomic approach: Recombinant expression and characterization. *Int. J. Biol. Macromol.* **2018**, *119*, 393–401. [[CrossRef](#)] [[PubMed](#)]
53. Hu, Y.; Fu, C.; Huang, Y.; Yin, Y.; Cheng, G.; Lei, F.; Lu, N.A.; Li, J.; Ashforth, E.J.; Zhang, L.; et al. Novel lipolytic genes from the microbial metagenomic library of the South China Sea marine sediment. *FEMS Microbiol. Ecol.* **2010**, *72*, 228–237. [[CrossRef](#)]
54. Fu, C.; Hu, Y.; Xie, F.; Guo, H.; Ashforth, E.J.; Polyak, S.W.; Zhu, B.; Zhang, L. Molecular cloning and characterization of a new cold-active esterase from a deep-sea metagenomic library. *Appl. Microbiol. Biotechnol.* **2011**, *90*, 961–970. [[CrossRef](#)] [[PubMed](#)]
55. Peng, Q.; Zhang, X.; Shang, M.; Wang, X.; Wang, G.; Li, B.; Guan, G.; Li, Y.; Wang, Y. A novel esterase gene cloned from a metagenomic library from neritic sediments of the South China Sea. *Microb. Cell Fact.* **2011**, *10*, 95. [[CrossRef](#)] [[PubMed](#)]
56. Yu, E.Y.; Kwon, M.A.; Lee, M.; Oh, J.Y.; Choi, J.E.; Lee, J.Y.; Song, B.K.; Hahm, D.H.; Song, J.K. Isolation and characterization of cold-active family VIII esterases from an arctic soil metagenome. *Appl. Microbiol. Biotechnol.* **2011**, *90*, 573–581. [[CrossRef](#)]
57. Fan, X.; Liu, X.; Huang, R.; Liu, Y. Identification and characterization of a novel thermostable pyrethroid-hydrolyzing enzyme isolated through metagenomic approach. *Microb. Cell Fact.* **2012**, *11*, 33. [[CrossRef](#)] [[PubMed](#)]
58. Jiang, X.; Xu, X.; Huo, Y.; Wu, Y.; Zhu, X.; Zhang, X.; Wu, M. Identification and characterization of novel esterases from a deep-sea sediment metagenome. *Arch. Microbiol.* **2012**, *194*, 207–214. [[CrossRef](#)] [[PubMed](#)]
59. Sulaiman, S.; Yamato, S.; Kanaya, E.; Kim, J.J.; Koga, Y.; Takano, K.; Kanaya, S. Isolation of a novel cutinase homolog with polyethylene terephthalate-degrading activity from leaf-branch compost by using a metagenomic approach. *Appl. Environ. Microbiol.* **2012**, *78*, 1556–1562. [[CrossRef](#)] [[PubMed](#)]
60. Sahoo, R.K.; Kumar, M.; Sukla, L.B.; Subudhi, E. Bioprospecting hot spring metagenome: Lipase for the production of biodiesel. *Environ. Sci. Pollut. Res.* **2017**, *24*, 3802–3809. [[CrossRef](#)] [[PubMed](#)]
61. Sahoo, R.K.; Das, A.; Sahoo, K.; Sahu, A.; Subudhi, E. Characterization of novel metagenomic-derived lipase from Indian hot spring. *Int. Microbiol.* **2020**, *23*, 233–240. [[CrossRef](#)] [[PubMed](#)]
62. Yang, Q.; Zhang, M.; Zhang, M.; Wang, C.; Liu, Y.; Fan, X.; Li, H. Characterization of a novel, cold-adapted, and thermostable laccase-like enzyme with high tolerance for organic solvents and salt and potent dye decolorization ability, derived from a marine metagenomic library. *Front. Microbiol.* **2018**, *9*, 2998. [[CrossRef](#)] [[PubMed](#)]
63. Chen, R.; Li, C.; Pei, X.; Wang, Q.; Yin, X.; Xie, T. Isolation an aldehyde dehydrogenase gene from Metagenomics based on semi-nest touch-down PCR. *Indian J. Microbiol.* **2014**, *54*, 74–79. [[CrossRef](#)] [[PubMed](#)]
64. Itoh, N.; Isotani, K.; Makino, Y.; Kato, M.; Kitayama, K.; Ishimota, T. PCR-based amplification and heterologous expression of *Pseudomonas* alcohol dehydrogenase genes from the soil metagenome for biocatalysis. *Enzym. Microb. Technol.* **2014**, *55*, 140–150. [[CrossRef](#)] [[PubMed](#)]
65. Itoh, N.; Kariya, S.; Kurokawa, J. Efficient PCR-based amplification of diverse alcohol dehydrogenase genes from metagenomes for improving biocatalysis: Screening of gene-specific amplicons from metagenomes. *Appl. Environ. Microbiol.* **2014**, *80*, 6280–6289. [[CrossRef](#)] [[PubMed](#)]
66. Neveu, J.; Regeard, C.; Dubow, M.S. Isolation and characterization of two serine proteases from metagenomic libraries of the Gobi and Death Valley deserts. *Appl. Microbiol. Biotechnol.* **2011**, *91*, 635–644. [[CrossRef](#)] [[PubMed](#)]
67. Singh, R.; Chopra, C.; Gupta, V.K.; Akhlaq, B.; Verma, V.; Rasool, S. Purification and characterization of CHpro1, a thermotolerant, alkali-stable and oxidation-resisting protease of Chumathang hot spring. *Sci. Bull.* **2015**, *60*, 1252–1260. [[CrossRef](#)]
68. Chen, W.; Zeng, Y.; Zheng, L.; Liu, W.; Lyu, Q. Discovery and characterization of a novel protease from the Antarctic soil. *Process Biochem.* **2021**, *111*, 270–277. [[CrossRef](#)]
69. Kanaya, E.; Sakabe, T.; Nguyen, N.T.; Koikeda, S.; Koga, Y.; Takano, K.; Kanaya, S. Cloning of the RNase H genes from a metagenomic DNA library: Identification of a new type 1 RNase H without a typical active-site motif. *J. Appl. Microbiol.* **2010**, *109*, 974–983. [[CrossRef](#)] [[PubMed](#)]
70. Bhat, A.; Riyaz-UI-Hassan, S.; Srivastava, N.; Johri, S. Molecular cloning of rhodanese gene from soil metagenome of cold desert of North-West Himalayas: Sequence and structural features of the rhodanese enzyme. *3 Biotech* **2015**, *5*, 513–521. [[CrossRef](#)] [[PubMed](#)]
71. Silva-Portela, R.C.B.; Carvalho, F.M.; Pereira, C.P.M.; De Souza-Pinto, N.C.; Modesti, M.; Fuchs, R.P.; Agnez-Lima, L.F. ExoMeg1: A new exonuclease from metagenomic library. *Sci. Rep.* **2016**, *6*, 19712. [[CrossRef](#)] [[PubMed](#)]
72. Liu, J.; Jia, Z.; Li, S.; Li, Y.; You, Q.; Zhang, C.; Zheng, X.; Xiong, G.; Zhao, J.; Qi, C.; et al. Identification and characterization of a chitin deacetylase from a metagenomic library of deep-sea sediments of the Arctic Ocean. *Gene* **2016**, *590*, 79–84. [[CrossRef](#)]
73. Agarwal, N.; Singh, S.P. A novel trehalose synthase for the production of trehalose and trehalulose. *Microbiol. Spectr.* **2021**, *9*, e01333-21. [[CrossRef](#)] [[PubMed](#)]
74. Perfumo, A.; Banat, I.M.; Marchant, R. Going green and cold: Biosurfactants from low-temperature environments to biotechnology applications. *Trends Biotechnol.* **2018**, *36*, 277–289. [[CrossRef](#)]
75. Morton, B.; Blackmore, G. South China Sea. *Mar. Pollut. Bull.* **2001**, *42*, 1236–1263. [[CrossRef](#)] [[PubMed](#)]
76. Pacchioni, R.G.; Carvalho, F.M.; Thompson, C.E.; Faustino, A.L.F.; Nicolini, F.; Pereira, T.S.; Silva, R.C.B.; Cantão, M.E.; Gerber, A.; Ana, T.R.; et al. Taxonomic and functional profiles of soil samples from Atlantic forest and Caatinga biomes in northeastern Brazil. *Microbiologyopen* **2014**, *3*, 299–315. [[CrossRef](#)] [[PubMed](#)]
77. DeCastro, M.E.; Rodríguez-Belmonte, E.; González-Siso, M.I. Metagenomics of thermophiles with a focus on discovery of novel thermozymes. *Front. Microbiol.* **2016**, *7*, 1521. [[CrossRef](#)] [[PubMed](#)]

78. Sánchez, Ó.J.; Ospina, D.A.; Montoya, S. Compost supplementation with nutrients and microorganisms in composting process. *Waste Manag.* **2017**, *69*, 136–153. [[CrossRef](#)] [[PubMed](#)]
79. Madhuri, R.J.; Saraswathi, M.; Gowthami, K.; Bhargavi, M.; Divya, Y.; Deepika, V. Recent approaches in the production of novel enzymes from environmental samples by enrichment culture and metagenomic approach. In *Recent Developments in Applied Microbiology and Biochemistry*; Buddolla, V., Ed.; Elsevier: Amsterdam, The Netherlands, 2019; pp. 251–262.
80. Wang, K.; Li, G.; Yu, S.Q.; Zhang, C.T.; Liu, Y.H. A novel metagenome-derived β -galactosidase: Gene cloning, overexpression, purification and characterization. *Appl. Microbiol. Biotechnol.* **2010**, *88*, 155–165. [[CrossRef](#)]
81. Cheng, J.; Romantsov, T.; Engel, K.; Doxey, A.C.; Rose, D.R.; Neufeld, J.D.; Charles, T.C. Functional metagenomics reveals novel β -galactosidases not predictable from gene sequences. *PLoS ONE* **2017**, *12*, e0172545. [[CrossRef](#)] [[PubMed](#)]
82. Allgaier, M.; Reddy, A.; Park, J.I.; Ivanova, N.; D’Haeseleer, P.; Lowry, S.; Sapra, R.; Hazen, T.C.; Simmons, B.A.; Vandergheynst, J.S.; et al. Targeted discovery of glycoside hydrolases from a switchgrass-adapted compost community. *PLoS ONE* **2010**, *5*, e8812. [[CrossRef](#)] [[PubMed](#)]
83. Alvarez, T.M.; Paiva, J.H.; Ruiz, D.M.; Cairo, J.P.L.F.; Pereira, I.O.; Paixão, D.A.A.; De Almeida, R.F.; Tonoli, C.C.C.; Ruller, R.; Santos, C.R.; et al. Structure and function of a novel cellulase 5 from sugarcane soil metagenome. *PLoS ONE* **2013**, *8*, e83635. [[CrossRef](#)]
84. Jiang, C.; Li, S.X.; Luo, F.F.; Jin, K.; Wang, Q.; Hao, Z.Y.; Wu, L.L.; Zhao, G.C.; Ma, G.F.; Shen, P.H.; et al. Biochemical characterization of two novel β -glucosidase genes by metagenome expression cloning. *Bioresour. Technol.* **2011**, *102*, 3272–3278. [[CrossRef](#)]
85. Liu, J.; Liu, W.D.; Zhao, X.L.; Shen, W.J.; Cao, H.; Cui, Z.L. Cloning and functional characterization of a novel endo- β -1,4-glucanase gene from a soil-derived metagenomic library. *Appl. Microbiol. Biotechnol.* **2011**, *89*, 1083–1092. [[CrossRef](#)] [[PubMed](#)]
86. Xiang, L.; Li, A.; Tian, C.; Zhou, Y.; Zhang, G.; Ma, Y. Identification and characterization of a new acid-stable endoglucanase from a metagenomic library. *Protein Expr. Purif.* **2014**, *102*, 20–26. [[CrossRef](#)] [[PubMed](#)]
87. Pandey, S.; Gulati, S.; Goyal, E.; Singh, S.; Kumar, K.; Nain, L.; Saxena, A.K. Construction and screening of metagenomic library derived from soil for β -1, 4-endoglucanase gene. *Biocatal. Agric. Biotechnol.* **2016**, *5*, 186–192. [[CrossRef](#)]
88. Yang, C.; Xia, Y.; Qu, H.; Li, A.D.; Liu, R.; Wang, Y.; Zhang, T. Discovery of new cellulases from the metagenome by a metagenomics-guided strategy. *Biotechnol. Biofuels* **2016**, *9*, 138. [[CrossRef](#)] [[PubMed](#)]
89. Meneses, C.; Silva, B.; Medeiros, B.; Serrato, R.; Johnston-Monje, D. A metagenomic advance for the cloning and characterization of a cellulase from red rice crop residues. *Molecules* **2016**, *21*, 831. [[CrossRef](#)]
90. Pimentel, A.C.; Ematsu, G.C.G.; Liberato, M.V.; Paixão, D.A.A.; Franco Cairo, J.P.L.; Mandelli, F.; Tramontina, R.; Gandin, C.A.; de Oliveira Neto, M.; Squina, F.M.; et al. Biochemical and biophysical properties of a metagenome-derived GH5 endoglucanase displaying an unconventional domain architecture. *Int. J. Biol. Macromol.* **2017**, *99*, 384–393. [[CrossRef](#)] [[PubMed](#)]
91. Lee, J.P.; Lee, H.W.; Na, H.B.; Lee, J.H.; Hong, Y.J.; Jeon, J.M.; Kwon, E.J.; Kim, S.K.; Kim, H. Characterization of truncated endo- β -1,4-glucanases from a compost metagenomic library and their saccharification potentials. *Int. J. Biol. Macromol.* **2018**, *115*, 554–562. [[CrossRef](#)]
92. Wierzbicka-Woś, A.; Henneberger, R.; Batista-García, R.A.; Martínez-Ávila, L.; Jackson, S.A.; Kennedy, J.; Dobson, A.D.W. Biochemical characterization of a novel monospecific endo- β -1,4-glucanase belonging to GH family 5 from a rhizosphere metagenomic library. *Front. Microbiol.* **2019**, *10*, 1342. [[CrossRef](#)] [[PubMed](#)]
93. Aymé, L.; Hébert, A.; Henrissat, B.; Lombard, V.; Franche, N.; Perret, S.; Jourdir, E.; Heiss-Blanquet, S. Characterization of three bacterial glycoside hydrolase family 9 endoglucanases with different modular architectures isolated from a compost metagenome. *Biochim. Biophys. Acta Gen. Subj.* **2021**, *1865*, 129848. [[CrossRef](#)] [[PubMed](#)]
94. Zhou, Y.; Wang, X.; Wei, W.; Xu, J.; Wang, W.; Xie, Z.; Zhang, Z.; Jiang, H.; Wang, Q.; Wei, C. A novel efficient β -glucanase from a paddy soil microbial metagenome with versatile activities. *Biotechnol. Biofuels* **2016**, *9*, 36. [[CrossRef](#)] [[PubMed](#)]
95. Hjort, K.; Bergström, M.; Adesina, M.F.; Jansson, J.K.; Smalla, K.; Sjöling, S. Chitinase genes revealed and compared in bacterial isolates, DNA extracts and a metagenomic library from a phytopathogen-suppressive soil. *FEMS Microbiol. Ecol.* **2010**, *71*, 197–207. [[CrossRef](#)] [[PubMed](#)]
96. Hjort, K.; Presti, I.; Elväng, A.; Marinelli, F.; Sjöling, S. Bacterial chitinase with phytopathogen control capacity from suppressive soil revealed by functional metagenomics. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 2819–2828. [[CrossRef](#)] [[PubMed](#)]
97. Cretoiu, M.S.; Berini, F.; Kielak, A.M.; Marinelli, F.; van Elsas, J.D. A novel salt-tolerant chitobiosidase discovered by genetic screening of a metagenomic library derived from chitin-amended agricultural soil. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 8199–8215. [[CrossRef](#)]
98. Stöveken, J.; Singh, R.; Kolkenbrock, S.; Zakrzewski, M.; Wibberg, D.; Eikmeyer, F.G.; Pühler, A.; Schlüter, A.; Moerschbacher, B.M. Successful heterologous expression of a novel chitinase identified by sequence analyses of the metagenome from a chitin-enriched soil sample. *J. Biotechnol.* **2015**, *201*, 60–68. [[CrossRef](#)]
99. Thimoteo, S.S.; Glogauer, A.; Faoro, H.; de Souza, E.M.; Huergo, L.F.; Moerschbacher, B.M.; Pedrosa, F.O. A broad pH range and processive chitinase from a metagenome library. *Braz. J. Med. Biol. Res.* **2017**, *50*, e5658. [[CrossRef](#)] [[PubMed](#)]
100. Delavat, F.; Phalip, V.; Forster, A.; Plewniak, F.; Lett, M.C.; Liévremont, D. Amylases without known homologues discovered in an acid mine drainage: Significance and impact. *Sci. Rep.* **2012**, *2*, 354. [[CrossRef](#)] [[PubMed](#)]

101. Soares, F.L.; Marcon, J.; Pereira e Silva, M.D.C.; Khakhum, N.; Cerdeira, L.T.; Ottoni, J.R.; Domingos, D.F.; Taketani, R.G.; De Oliveira, V.M.; Lima, A.O.D.S.; et al. A Novel Multifunctional β -N-Acetylhexosaminidase Revealed through Metagenomics of an Oil-Spilled Mangrove. *Bioengineering* **2017**, *4*, 62. [[CrossRef](#)]
102. Jeong, Y.S.; Na, H.B.; Kim, S.K.; Kim, Y.H.; Kwon, E.J.; Kim, J.; Yun, H.D.; Lee, J.K.; Kim, H. Characterization of Xyn10], a novel family 10 xylanase from a compost metagenomic library. *Appl. Biochem. Biotechnol.* **2012**, *166*, 1328–1339. [[CrossRef](#)] [[PubMed](#)]
103. Alvarez, T.M.; Goldbeck, R.; dos Santos, C.R.; Paixão, D.A.A.; Gonçalves, T.A.; Franco Cairo, J.P.L.; Almeida, R.F.; de Oliveira Pereira, I.; Jackson, G.; Cota, J.; et al. Development and biotechnological application of a novel endoxylanase family GH10 identified from sugarcane soil metagenome. *PLoS ONE* **2013**, *8*, e70014. [[CrossRef](#)] [[PubMed](#)]
104. Ellilä, S.; Bromann, P.; Nyssönen, M.; Itävaara, M.; Koivula, A.; Paulin, L.; Kruus, K. Cloning of novel bacterial xylanases from lignocellulose-enriched compost metagenomic libraries. *AMB Express* **2019**, *9*, 124. [[CrossRef](#)] [[PubMed](#)]
105. Wang, J.; Liang, J.; Li, Y.; Tian, L.; Wei, Y. Characterization of efficient xylanases from industrial-scale pulp and paper wastewater treatment microbiota. *AMB Express* **2021**, *11*, 19. [[CrossRef](#)]
106. Ndata, K.; Nevondo, W.; Cekuse, B.; van Zyl, L.J.; Trindade, M. Characterization of a highly xylose tolerant β -xylosidase isolated from high temperature horse manure compost. *BMC Biotechnol.* **2021**, *21*, 61. [[CrossRef](#)] [[PubMed](#)]
107. Kwon, E.J.; Jeong, Y.S.; Kim, Y.H.; Kim, S.K.; Na, H.B.; Kim, J.; Yun, H.D.; Kim, H. Construction of a metagenomic library from compost and screening of cellulase- and xylanase-positive clones. *J. Appl. Biol. Chem.* **2010**, *53*, 702–708. [[CrossRef](#)]
108. Gladden, J.M.; Allgaier, M.; Miller, C.S.; Hazen, T.C.; VanderGheynst, J.S.; Hugenholtz, P.; Simmons, B.A.; Singer, S.W. Glycoside hydrolase activities of thermophilic bacterial consortia adapted to switchgrass. *Appl. Environ. Microbiol.* **2011**, *77*, 5804–5812. [[CrossRef](#)] [[PubMed](#)]
109. Dougherty, M.J.; D'haeseleer, P.; Hazen, T.C.; Simmons, B.A.; Adams, P.D.; Hadi, M.Z. Glycoside hydrolases from a targeted compost metagenome, activity-screening and functional characterization. *BMC Biotechnol.* **2012**, *12*, 38. [[CrossRef](#)] [[PubMed](#)]
110. Kanokratana, P.; Eurwilaichitr, L.; Pootanakit, K.; Champreda, V. Identification of glycosyl hydrolases from a metagenomic library of microflora in sugarcane bagasse collection site and their cooperative action on cellulose degradation. *J. Biosci. Bioeng.* **2015**, *119*, 384–391. [[CrossRef](#)] [[PubMed](#)]
111. Berlemont, R.; Pipers, D.; Delsaute, M.; Angiono, F.; Feller, G.; Galleni, M.; Power, P. Exploring the Antarctic soil metagenome as a source of novel cold-adapted enzymes and genetic mobile elements. *Rev. Argent. Microbiol.* **2011**, *43*, 94–103. [[CrossRef](#)] [[PubMed](#)]
112. Stroobants, A.; Portetelle, D.; Vandenberg, M. New carbohydrate-active enzymes identified by screening two metagenomic libraries derived from the soil of a winter wheat field. *J. Appl. Microbiol.* **2014**, *117*, 1045–1055. [[CrossRef](#)]
113. Liaw, R.B.; Cheng, M.P.; Wu, M.C.; Lee, C.Y. Use of metagenomic approaches to isolate lipolytic genes from activated sludge. *Bioresour. Technol.* **2010**, *101*, 8323–8329. [[CrossRef](#)] [[PubMed](#)]
114. Kim, Y.H.; Kwon, E.J.; Kim, S.K.; Jeong, Y.S.; Kim, J.; Yun, H.D.; Kim, H. Molecular cloning and characterization of a novel family VIII alkaline esterase from a compost metagenomic library. *Biochem. Biophys. Res. Commun.* **2010**, *393*, 45–49. [[CrossRef](#)] [[PubMed](#)]
115. Lee, M.H.; Hong, K.S.; Malhotra, S.; Park, J.H.; Hwang, E.C.; Choi, H.K.; Kim, Y.S.; Tao, W.; Lee, S.W. A new esterase EstD2 isolated from plant rhizosphere soil metagenome. *Appl. Microbiol. Biotechnol.* **2010**, *88*, 1125–1134. [[CrossRef](#)]
116. Sang, S.L.; Li, G.; Hu, X.P.; Liu, Y.H. Molecular cloning, overexpression and characterization of a novel feruloyl esterase from a soil metagenomic library. *J. Mol. Microbiol. Biotechnol.* **2011**, *20*, 196–203. [[CrossRef](#)] [[PubMed](#)]
117. Pereira, M.R.; Mercaldi, G.F.; Maester, T.C.; Balan, A.; De Macedo Lemos, E.G. Est16, a new esterase isolated from a metagenomic library of a microbial consortium specializing in diesel oil degradation. *PLoS ONE* **2015**, *10*, e0133723. [[CrossRef](#)]
118. Ohlhoff, C.W.; Kirby, B.M.; Van Zyl, L.; Mutepefa, D.L.R.; Casanueva, A.; Huddy, R.J.; Bauer, R.; Cowan, D.A.; Tuffin, M. An unusual feruloyl esterase belonging to family VIII esterases and displaying a broad substrate range. *J. Mol. Catal. B Enzym.* **2015**, *118*, 79–88. [[CrossRef](#)]
119. Lee, H.W.; Jung, W.K.; Kim, Y.H.; Ryu, B.H.; Doohun Kim, T.; Kim, J.; Kim, H. Characterization of a novel alkaline family viii esterase with S-enantiomer preference from a compost metagenomic library. *J. Microbiol. Biotechnol.* **2016**, *26*, 315–325. [[CrossRef](#)]
120. Dukunde, A.; Schneider, D.; Lu, M.; Brady, S.; Daniel, R. A novel, versatile family IV carboxylesterase exhibits high stability and activity in a broad pH spectrum. *Biotechnol. Lett.* **2017**, *39*, 577–587. [[CrossRef](#)] [[PubMed](#)]
121. Lu, M.; Dukunde, A.; Daniel, R. Biochemical profiles of two thermostable and organic solvent-tolerant esterases derived from a compost metagenome. *Appl. Microbiol. Biotechnol.* **2019**, *103*, 3421–3437. [[CrossRef](#)] [[PubMed](#)]
122. Yao, J.; Gui, L.; Yin, S. A novel esterase from a soil metagenomic library displaying a broad substrate range. *AMB Express* **2021**, *11*, 38. [[CrossRef](#)] [[PubMed](#)]
123. Yan, Z.; Ding, L.; Zou, D.; Wang, L.; Tan, Y.; Guo, S.; Zhang, Y.; Xin, Z. Identification and characterization of a novel carboxylesterase EstQ7 from a soil metagenomic library. *Arch. Microbiol.* **2021**, *203*, 4113–4125. [[CrossRef](#)] [[PubMed](#)]
124. Yan, Z.; Ding, L.; Zou, D.; Qiu, J.; Shao, Y.; Sun, S.; Li, L.; Xin, Z. Characterization of a novel carboxylesterase with catalytic activity toward di(2-ethylhexyl) phthalate from a soil metagenomic library. *Sci. Total Environ.* **2021**, *785*, 147260. [[CrossRef](#)]
125. Park, J.E.; Jeong, G.S.; Lee, H.W.; Kim, H. Biochemical characterization of a family IV esterase with R-form enantioselectivity from a compost metagenomic library. *Appl. Biol. Chem.* **2021**, *64*, 81. [[CrossRef](#)]
126. Park, J.E.; Jeong, G.S.; Lee, H.W.; Kim, H. Molecular characterization of novel family iv and viii esterases from a compost metagenomic library. *Microorganisms* **2021**, *9*, 1614. [[CrossRef](#)] [[PubMed](#)]

127. Park, J.E.; Jeong, G.S.; Lee, H.W.; Kim, S.K.; Kim, J.; Kim, H. Characterization of a novel family iv esterase containing a predicted czco domain and a family v esterase with broad substrate specificity from an oil-polluted mud flat metagenomic library. *Appl. Sci.* **2021**, *11*, 5905. [[CrossRef](#)]
128. O'Mahony, M.M.; Henneberger, R.; Selvin, J.; Kennedy, J.; Doohan, F.; Marchesi, J.R.; Dobson, A.D.W. Inhibition of the growth of *Bacillus subtilis* DSM10 by a newly discovered antibacterial protein from the soil metagenome. *Bioengineered* **2015**, *6*, 89–98. [[CrossRef](#)]
129. Glogauer, A.; Martini, V.P.; Faoro, H.; Couto, G.H.; Müller-Santos, M.; Monteiro, R.A.; Mitchell, D.A.; de Souza, E.M.; Pedrosa, F.O.; Krieger, N. Identification and characterization of a new true lipase isolated through metagenomic approach. *Microb. Cell Fact.* **2011**, *10*, 54. [[CrossRef](#)] [[PubMed](#)]
130. Zheng, J.; Liu, C.; Liu, L.; Jin, Q. Characterisation of a thermo-alkali-stable lipase from oil-contaminated soil using a metagenomic approach. *Syst. Appl. Microbiol.* **2013**, *36*, 197–204. [[CrossRef](#)] [[PubMed](#)]
131. Kim, H.J.; Jeong, Y.S.; Jung, W.K.; Kim, S.K.; Lee, H.W.; Kahng, H.Y.; Kim, J.; Kim, H. Characterization of novel family IV esterase and family I.3 lipase from an oil-polluted mud flat metagenome. *Mol. Biotechnol.* **2015**, *57*, 781–792. [[CrossRef](#)]
132. Yao, J.; Fan, X.J.; Lu, Y.; Liu, Y.H. Isolation and characterization of a novel tannase from a metagenomic library. *J. Agric. Food Chem.* **2011**, *59*, 3812–3818. [[CrossRef](#)]
133. Nacke, H.; Will, C.; Herzog, S.; Nowka, B.; Engelhaupt, M.; Daniel, R. Identification of novel lipolytic genes and gene families by screening of metagenomic libraries derived from soil samples of the German Biodiversity Exploratories. *FEMS Microbiol. Ecol.* **2011**, *78*, 188–201. [[CrossRef](#)] [[PubMed](#)]
134. Stroobants, A.; Martin, R.; Roosens, L.; Portetelle, D.; Vandenbol, M. New lipolytic enzymes identified by screening two metagenomic libraries derived from the soil of a winter wheat field. *Biotechnol. Agron. Soc. Environ.* **2015**, *19*, 125–131.
135. Kimura, N.; Kamagata, Y. A thermostable bilirubin-oxidizing enzyme from activated sludge isolated by a metagenomic approach. *Microbes Environ.* **2016**, *31*, 435–441. [[CrossRef](#)] [[PubMed](#)]
136. Ou, Q.; Liu, Y.; Deng, J.; Chen, G.; Yang, Y.; Shen, P.; Wu, B.; Jiang, C. A novel d-amino acid oxidase from a contaminated agricultural soil metagenome and its characterization. *Antonie Leeuwenhoek Int. J. Gen. Mol. Microbiol.* **2015**, *107*, 1615–1623. [[CrossRef](#)] [[PubMed](#)]
137. Kimura, N.; Sakai, K.; Nakamura, K. Isolation and characterization of a 4-nitrotoluene-oxidizing enzyme from activated sludge by a metagenomic approach. *Microbes Environ.* **2010**, *25*, 133–139. [[CrossRef](#)] [[PubMed](#)]
138. Singh, A.; Singh Chauhan, N.; Thulasiram, H.V.; Taneja, V.; Sharma, R. Identification of two flavin monooxygenases from an effluent treatment plant sludge metagenomic library. *Bioresour. Technol.* **2010**, *101*, 8481–8484. [[CrossRef](#)] [[PubMed](#)]
139. Chemerys, A.; Pelletier, E.; Cruaud, C.; Martin, F.; Violet, F.; Jouanneau, Y. Characterization of novel polycyclic aromatic hydrocarbon dioxygenases from the bacterial metagenomic DNA of a contaminated soil. *Appl. Environ. Microbiol.* **2014**, *80*, 6591–6600. [[CrossRef](#)] [[PubMed](#)]
140. Lu, Y.; Yu, Y.; Zhou, R.; Sun, W.; Dai, C.; Wan, P.; Zhang, L.; Hao, D.; Ren, H. Cloning and characterisation of a novel 2,4-dichlorophenol hydroxylase from a metagenomic library derived from polychlorinated biphenyl-contaminated soil. *Biotechnol. Lett.* **2011**, *33*, 1159–1167. [[CrossRef](#)] [[PubMed](#)]
141. Tan, H.; Mooij, M.J.; Barret, M.; Hegarty, P.M.; Harrington, C.; Dobson, A.D.W.; O'Gara, F. Identification of novel phytase genes from an agricultural soil-derived metagenome. *J. Microbiol. Biotechnol.* **2014**, *24*, 113–118. [[CrossRef](#)] [[PubMed](#)]
142. Kim, N.H.; Park, J.H.; Chung, E.; So, H.A.; Lee, M.H.; Kim, J.C.; Hwang, E.C.; Lee, S.W. Characterization of a soil metagenome-derived gene encoding wax ester synthase. *J. Microbiol. Biotechnol.* **2016**, *26*, 248–254. [[CrossRef](#)] [[PubMed](#)]
143. Jiang, L.; Lin, M.; Zhang, Y.; Li, Y.; Xu, X.; Li, S.; Huang, H. Identification and characterization of a novel trehalose synthase gene derived from saline-alkali soil metagenomes. *PLoS ONE* **2013**, *8*, e77437. [[CrossRef](#)] [[PubMed](#)]
144. Wang, S.D.; Guo, G.S.; Li, L.; Cao, L.C.; Tong, L.; Ren, G.H.; Liu, Y.H. Identification and characterization of an unusual glycosyltransferase-like enzyme with β -galactosidase activity from a soil metagenomic library. *Enzym. Microb. Technol.* **2014**, *57*, 26–35. [[CrossRef](#)] [[PubMed](#)]
145. Colin, P.Y.; Kintsjes, B.; Gielen, F.; Miton, C.M.; Fischer, G.; Mohamed, M.F.; Hyvönen, M.; Morgavi, D.P.; Janssen, D.B.; Hollfelder, F. Ultrahigh-throughput discovery of promiscuous enzymes by picodroplet functional metagenomics. *Nat. Commun.* **2015**, *6*, 10008. [[CrossRef](#)] [[PubMed](#)]
146. Ajibade, F.O.; Adelodun, B.; Lasisi, K.H.; Fadare, O.O.; Ajibade, T.F.; Nwogwu, N.A.; Sulaymon, I.D.; Ugya, A.Y.; Wang, H.C.; Wang, A. Chapter 25—Environmental pollution and their socioeconomic impacts. In *Microbe Mediated Remediation of Environmental Contaminants*; Kumar, A., Singh, V.K., Singh, P., Mishra, V.K., Eds.; Woodhead Publishing: Cambridge, UK, 2021; pp. 321–354. ISBN 9780128211991.
147. Dindar, E.; Topaç Şağban, F.O.; Başkaya, H.S. Variations of soil enzyme activities in petroleum-hydrocarbon contaminated soil. *Int. Biodeterior. Biodegrad.* **2015**, *105*, 268–275. [[CrossRef](#)]
148. Waldhauser, F.; Schaff, D.; Richards, P.G.; Kim, W.-Y. Lop Nor Revisited: Underground nuclear explosion locations, 1976–1996, from double-difference analysis of regional and teleseismic data. *Bull. Seismol. Soc. Am.* **2004**, *94*, 1879–1889. [[CrossRef](#)]
149. Van Leeuwen, J.P.; Djukic, I.; Bloem, J.; Lehtinen, T.; Hemerik, L. Effects of land use on soil microbial biomass, activity and community structure at different soil depths in the Danube floodplain. *Eur. J. Soil Biol.* **2017**, *79*, 14–20. [[CrossRef](#)]

150. Souza, R.C.; Hungria, M.; Cantão, M.E.; Vasconcelos, A.T.R.; Nogueira, M.A.; Vicente, V.A. Metagenomic analysis reveals microbial functional redundancies and specificities in a soil under different tillage and crop-management regimes. *Appl. Soil Ecol.* **2015**, *86*, 106–112. [[CrossRef](#)]
151. Siles-Castellano, A.B.; López, M.J.; López-González, J.A.; Suárez-Estrella, F.; Jurado, M.M.; Estrella-González, M.J.; Moreno, J. Comparative analysis of phytotoxicity and compost quality in industrial composting facilities processing different organic wastes. *J. Clean. Prod.* **2020**, *252*, 119820. [[CrossRef](#)]
152. Bergmann, J.C.; Costa, O.Y.A.; Gladden, J.M.; Singer, S.; Heins, R.; D'haeseleer, P.; Simmons, B.A.; Quirino, B.F. Discovery of two novel β -glucosidases from an Amazon soil metagenomic library. *FEMS Microbiol. Lett.* **2014**, *351*, 147–155. [[CrossRef](#)] [[PubMed](#)]
153. Couto, G.H.; Glogauer, A.; Faoro, H.; Chubatsu, L.S.; Souza, E.M.; Pedrosa, F.O. Isolation of a novel lipase from a metagenomic library derived from mangrove sediment from the south Brazilian coast. *Genet. Mol. Res.* **2010**, *9*, 514–523. [[CrossRef](#)]
154. Ye, M.; Li, G.; Liang, W.Q.; Liu, Y.H. Molecular cloning and characterization of a novel metagenome-derived multicopper oxidase with alkaline laccase activity and highly soluble expression. *Appl. Microbiol. Biotechnol.* **2010**, *87*, 1023–1031. [[CrossRef](#)] [[PubMed](#)]
155. Li, G.; Jiang, Y.; Fan, X.J.; Liu, Y.H. Molecular cloning and characterization of a novel β -glucosidase with high hydrolyzing ability for soybean isoflavone glycosides and glucose-tolerance from soil metagenomic library. *Bioresour. Technol.* **2012**, *123*, 15–22. [[CrossRef](#)]
156. Mai, Z.; Su, H.; Yang, J.; Huang, S.; Zhang, S. Cloning and characterization of a novel GH44 family endoglucanase from mangrove soil metagenomic library. *Biotechnol. Lett.* **2014**, *36*, 1701–1709. [[CrossRef](#)] [[PubMed](#)]
157. Mai, Z.; Su, H.; Zhang, S. Isolation and characterization of a glycosyl hydrolase family 16 β -agarase from a mangrove soil metagenomic library. *Int. J. Mol. Sci.* **2016**, *17*, 1360. [[CrossRef](#)] [[PubMed](#)]
158. Bunterngsook, B.; Kanokratana, P.; Thongaram, T.; Tanapongpipat, S.; Uengwetwanit, T.; Rachdawong, S.; Vichitsoonthonkul, T.; Eurwilaichitr, L. Identification and characterization of lipolytic enzymes from a peat-swamp forest soil metagenome. *Biosci. Biotechnol. Biochem.* **2010**, *74*, 1848–1854. [[CrossRef](#)]
159. Tan, H.; Wu, X.; Xie, L.; Huang, Z.; Peng, W.; Gan, B. A novel phytase derived from an acidic peat-soil microbiome showing high stability under acidic plus pepsin conditions. *J. Mol. Microbiol. Biotechnol.* **2016**, *26*, 291–301. [[CrossRef](#)] [[PubMed](#)]
160. Berlemont, R.; Spee, O.; Delsaute, M.; Lara, Y.; Schuldes, J.; Simon, C.; Power, P.; Daniel, R.; Galleni, M. Novel organic solvent-tolerant esterase isolated by metagenomics: Insights into the lipase/esterase classification. *Rev. Argent. Microbiol.* **2013**, *45*, 3–12. [[PubMed](#)]
161. Gu, X.; Wang, S.; Wang, S.; Zhao, L.X.; Cao, M.; Feng, Z. Identification and characterization of two novel esterases from a metagenomic library. *Food Sci. Technol. Res.* **2015**, *21*, 649–657. [[CrossRef](#)]
162. Gomes-Pepe, E.S.; Sierra, E.G.M.; Pereira, M.R.; Castellane, T.C.L.; De Lemos, E.G.M. Bg10: A novel metagenomics alcohol-tolerant and glucose-stimulated gh1 β -glucosidase suitable for lactose-free milk preparation. *PLoS ONE* **2016**, *11*, e0167932. [[CrossRef](#)] [[PubMed](#)]
163. Tao, W.; Lee, M.H.; Yoon, M.Y.; Kim, J.C.; Malhotra, S.; Wu, J.; Hwang, E.C.; Lee, S.W. Characterization of two metagenome-derived esterases that reactivate chloramphenicol by counteracting chloramphenicol acetyltransferase. *J. Microbiol. Biotechnol.* **2011**, *21*, 1203–1210. [[CrossRef](#)]
164. Li, Z.; Li, X.; Liu, T.; Chen, S.; Liu, H.; Wang, H.; Li, K.; Song, Y.; Luo, X.; Zhao, J.; et al. The critical roles of exposed surface residues for the thermostability and halotolerance of a novel GH11 xylanase from the metagenomic library of a saline-alkaline soil. *Int. J. Biol. Macromol.* **2019**, *133*, 316–323. [[CrossRef](#)] [[PubMed](#)]
165. Istvan, P.; Souza, A.A.; Garay, A.V.; dos Santos, D.F.K.; de Oliveira, G.M.; Santana, R.H.; Lopes, F.A.C.; de Freitas, S.M.; Barbosa, J.A.R.G.; Krüger, R.H. Structural and functional characterization of a novel lipolytic enzyme from a Brazilian Cerrado soil metagenomic library. *Biotechnol. Lett.* **2018**, *40*, 1395–1406. [[CrossRef](#)]
166. dos Santos, D.F.K.; Istvan, P.; Noronha, E.F.; Quirino, B.F.; Krüger, R.H. New dioxygenase from metagenomic library from Brazilian soil: Insights into antibiotic resistance and bioremediation. *Biotechnol. Lett.* **2015**, *37*, 1809–1817. [[CrossRef](#)] [[PubMed](#)]
167. Zhao, X.; Liu, L.; Deng, Z.; Liu, S.; Yun, J.; Xiao, X.; Li, H. Screening, cloning, enzymatic properties of a novel thermostable cellulase enzyme, and its potential application on water hyacinth utilization. *Int. Microbiol.* **2021**, *24*, 337–349. [[CrossRef](#)]
168. Erich, S.; Kuschel, B.; Schwarz, T.; Ewert, J.; Böhmer, N.; Niehaus, F.; Eck, J.; Lutz-Wahl, S.; Stressler, T.; Fischer, L. Novel high-performance metagenome β -galactosidases for lactose hydrolysis in the dairy industry. *J. Biotechnol.* **2015**, *210*, 27–37. [[CrossRef](#)]
169. Lezyk, M.; Jers, C.; Kjaerulff, L.; Gotfredsen, C.H.; Mikkelsen, M.D.; Mikkelsen, J.D. Novel α -L-fucosidases from a soil metagenome for production of fucosylated human milk oligosaccharides. *PLoS ONE* **2016**, *11*, e0147438. [[CrossRef](#)]
170. Kim, S.Y.; Oh, D.B.; Kwon, O. Characterization of a lichenase isolated from soil metagenome. *J. Microbiol. Biotechnol.* **2014**, *24*, 1699–1706. [[CrossRef](#)] [[PubMed](#)]
171. Sathya, T.A.; Jacob, A.M.; Khan, M. Cloning and molecular modelling of pectin degrading glycosyl hydrolase of family 28 from soil metagenomic library. *Mol. Biol. Rep.* **2014**, *41*, 2645–2656. [[CrossRef](#)] [[PubMed](#)]
172. Mori, T.; Kamei, I.; Hirai, H.; Kondo, R. Identification of novel glycosyl hydrolases with cellulolytic activity against crystalline cellulose from metagenomic libraries constructed from bacterial enrichment cultures. *SpringerPlus* **2014**, *3*, 365. [[CrossRef](#)]
173. Chai, S.; Zhang, X.; Jia, Z.; Xu, X.; Zhang, Y.; Wang, S.; Feng, Z. Identification and characterization of a novel bifunctional cellulase/hemicellulase from a soil metagenomic library. *Appl. Microbiol. Biotechnol.* **2020**, *104*, 7563–7572. [[CrossRef](#)] [[PubMed](#)]

174. Faoro, H.; Glogauer, A.; Couto, G.H.; de Souza, E.M.; Rigo, L.U.; Cruz, L.M.; Monteiro, R.A.; de Oliveira Pedrosa, F. Characterization of a new Acidobacteria-derived moderately thermostable lipase from a Brazilian Atlantic Forest soil metagenome. *FEMS Microbiol. Ecol.* **2012**, *81*, 386–394. [[CrossRef](#)] [[PubMed](#)]
175. Lim, H.K.; Han, Y.J.; Hahm, M.S.; Park, S.Y.; Hwang, I.T. Isolation and characterization of a novel triolein selective lipase from soil environmental genes. *Microbiol. Biotechnol. Lett.* **2021**, *48*, 480–490. [[CrossRef](#)]
176. Jin, P.; Pei, X.; Du, P.; Yin, X.; Xiong, X.; Wu, H.; Zhou, X.; Wang, Q. Overexpression and characterization of a new organic solvent-tolerant esterase derived from soil metagenomic DNA. *Bioresour. Technol.* **2012**, *116*, 234–240. [[CrossRef](#)]
177. Biver, S.; Vandenbol, M. Characterization of three new carboxylic ester hydrolases isolated by functional screening of a forest soil metagenomic library. *J. Ind. Microbiol. Biotechnol.* **2013**, *40*, 191–200. [[CrossRef](#)]
178. Jeon, J.H.; Lee, H.S.; Lee, J.H.; Koo, B.S.; Lee, C.M.; Lee, S.H.; Kang, S.G.; Lee, J.H. A novel family VIII carboxylesterase hydrolysing third- and fourth-generation cephalosporins. *SpringerPlus* **2016**, *5*, 525. [[CrossRef](#)]
179. Park, J.M.; Won, S.M.; Kang, C.H.; Park, S.; Yoon, J.H. Characterization of a novel carboxylesterase belonging to family VIII hydrolyzing β -lactam antibiotics from a compost metagenomic library. *Int. J. Biol. Macromol.* **2020**, *164*, 4650–4661. [[CrossRef](#)]
180. Nagayama, H.; Sugawara, T.; Endo, R.; Ono, A.; Kato, H.; Ohtsubo, Y.; Nagata, Y.; Tsuda, M. Isolation of oxygenase genes for indigo-forming activity from an artificially polluted soil metagenome by functional screening using *Pseudomonas putida* strains as hosts. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 4453–4470. [[CrossRef](#)] [[PubMed](#)]
181. Biver, S.; Portetelle, D.; Vandenbol, M. Characterization of a new oxidant-stable serine protease isolated by functional metagenomics. *SpringerPlus* **2013**, *2*, 410. [[CrossRef](#)] [[PubMed](#)]
182. Stephens, G.L.; Slingo, J.M.; Rignot, E.; Reager, J.T.; Hakuba, M.Z.; Durack, P.J.; Worden, J.; Rocca, R. Earth's water reservoirs in a changing climate. *Proc. R. Soc. A Math. Phys. Eng. Sci.* **2020**, *476*, 20190458. [[CrossRef](#)]
183. Parages, M.L.; Gutiérrez-Barranquero, J.A.; Reen, F.J.; Dobson, A.D.W.; O'Gara, F. Integrated (Meta) genomic and synthetic biology approaches to develop new biocatalysts. *Mar. Drugs* **2016**, *14*, 62. [[CrossRef](#)]
184. Jiang, T.; Sun, S.; Chen, Y.; Qian, Y.; Guo, J.; Dai, R.; An, D. Microbial diversity characteristics and the influence of environmental factors in a large drinking-water source. *Sci. Total Environ.* **2021**, *769*, 144698. [[CrossRef](#)]
185. Kamble, P.; Vavilala, S.L. Discovering novel enzymes from marine ecosystems: A metagenomic approach. *Bot. Mar.* **2018**, *61*, 161–175. [[CrossRef](#)]
186. Fang, W.; Liu, J.; Hong, Y.; Peng, H.; Zhang, X.; Sun, B.; Xiao, Y. Cloning and characterization of a β -glucosidase from marine microbial metagenome with excellent glucose tolerance. *J. Microbiol. Biotechnol.* **2010**, *20*, 1351–1358. [[CrossRef](#)]
187. Wierzbicka-Woś, A.; Bartasun, P.; Cieśliński, H.; Kur, J. Cloning and characterization of a novel cold-active glycoside hydrolase family 1 enzyme with β -glucosidase, β -fucosidase and β -galactosidase activities. *BMC Biotechnol.* **2013**, *13*, 22. [[CrossRef](#)]
188. Toyama, D.; de Morais, M.A.B.; Ramos, F.C.; Zanphorlin, L.M.; Tonoli, C.C.C.; Balula, A.F.; de Miranda, F.P.; Almeida, V.M.; Marana, S.R.; Ruller, R.; et al. A novel β -glucosidase isolated from the microbial metagenome of Lake Poraquê (Amazon, Brazil). *Biochim. Biophys. Acta Prot. Proteom.* **2018**, *1866*, 569–579. [[CrossRef](#)] [[PubMed](#)]
189. Mohamed, Y.M.; Ghazy, M.A.; Sayed, A.; Ouf, A.; El-Dorry, H.; Siam, R. Isolation and characterization of a heavy metal-resistant, thermophilic esterase from a Red Sea Brine Pool. *Sci. Rep.* **2013**, *3*, 3358. [[CrossRef](#)] [[PubMed](#)]
190. Fang, Z.; Li, J.; Wang, Q.; Fang, W.; Peng, H.; Zhang, X.; Xiao, Y. A novel esterase from a marine metagenomic library exhibiting salt tolerance ability. *J. Microbiol. Biotechnol.* **2014**, *24*, 771–780. [[CrossRef](#)] [[PubMed](#)]
191. López-López, O.; Knapik, K.; Cerdán, M.E.; González-Siso, M.I. Metagenomics of an alkaline hot spring in Galicia (Spain): Microbial diversity analysis and screening for novel lipolytic enzymes. *Front. Microbiol.* **2015**, *6*, 1291. [[CrossRef](#)]
192. Fang, Z.; Li, T.; Wang, Q.; Zhang, X.; Peng, H.; Fang, W.; Hong, Y.; Ge, H.; Xiao, Y. A bacterial laccase from marine microbial metagenome exhibiting chloride tolerance and dye decolorization ability. *Appl. Microbiol. Biotechnol.* **2011**, *89*, 1103–1110. [[CrossRef](#)] [[PubMed](#)]
193. Sayed, A.; Ghazy, M.A.; Ferreira, A.J.S.; Setubal, J.C.; Chambergo, F.S.; Ouf, A.; Adel, M.; Dawe, A.S.; Archer, J.A.C.; Bajic, V.B.; et al. A novel mercuric reductase from the unique deep brine environment of Atlantis II in the Red Sea. *J. Biol. Chem.* **2014**, *289*, 1675–1687. [[CrossRef](#)] [[PubMed](#)]
194. Badia, E.A.; Sayed, A.A.; Maged, M.; Fouad, W.M.; Said, M.M.; Esmat, A.Y. A novel thermostable and halophilic thioredoxin reductase from the Red Sea Atlantis II hot brine pool. *PLoS ONE* **2019**, *14*, e0217565. [[CrossRef](#)] [[PubMed](#)]
195. Witte, B.; John, D.; Wawrik, B.; Paul, J.H.; Dayan, D.; Robert Tabita, F. Functional prokaryotic rubisCO from an oceanic metagenomic library. *Appl. Environ. Microbiol.* **2010**, *76*, 2997–3003. [[CrossRef](#)] [[PubMed](#)]
196. Jiang, C.; Wu, L.L.; Zhao, G.C.; Shen, P.H.; Jin, K.; Hao, Z.Y.; Li, S.X.; Ma, G.F.; Luo, F.F.; Hu, G.Q.; et al. Identification and characterization of a novel fumarase gene by metagenome expression cloning from marine microorganisms. *Microb. Cell Fact.* **2010**, *9*, 91. [[CrossRef](#)]
197. Sonbol, S.A.; Ferreira, A.J.S.; Siam, R. Red Sea Atlantis II brine pool nitrilase with unique thermostability profile and heavy metal tolerance. *BMC Biotechnol.* **2016**, *16*, 14. [[CrossRef](#)]
198. Elbeheri, A.H.A.; Leak, D.J.; Siam, R. Novel thermostable antibiotic resistance enzymes from the Atlantis II Deep Red Sea brine pool. *Microb. Biotechnol.* **2017**, *10*, 189–202. [[CrossRef](#)]
199. Sobat, M.; Asad, S.; Kabiri, M.; Mehrshad, M. Metagenomic discovery and functional validation of L-asparaginases with anti-leukemic effect from the Caspian Sea. *iScience* **2021**, *24*, 101973. [[CrossRef](#)]
200. Shu, W.S.; Huang, L.N. Microbial diversity in extreme environments. *Nat. Rev. Microbiol.* **2021**, *20*, 219–235. [[CrossRef](#)] [[PubMed](#)]

201. Poli, A.; Finore, I.; Romano, I.; Gioiello, A.; Lama, L.; Nicolaus, B. Microbial diversity in extreme marine habitats and their biomolecules. *Microorganisms* **2017**, *5*, 25. [[CrossRef](#)]
202. Ojaveer, H.; Jaanus, A.; Mackenzie, B.R.; Martin, G.; Olenin, S.; Radziejewska, T.; Telesh, I.; Zettler, M.L.; Zaiko, A. Status of biodiversity in the Baltic Sea. *PLoS ONE* **2010**, *5*, e12467. [[CrossRef](#)] [[PubMed](#)]
203. Sass, A.M.; Sass, H.; Coolen, M.J.L.; Cypionka, H.; Overmann, J. Microbial communities in the chemocline of a hypersaline deep-sea basin (Urania Basin, Mediterranean Sea). *Appl. Environ. Microbiol.* **2001**, *67*, 5392–5402. [[CrossRef](#)]
204. Borah, P.; Kumar, M.; Devi, P. Types of inorganic pollutants: Metals/metalloids, acids, and organic forms. In *Inorganic Pollutants in Water*; Devi, P., Singh, P., Kansal, S.K., Eds.; Elsevier: Amsterdam, The Netherlands, 2020; pp. 17–31. ISBN 9780128189658.
205. Ouyang, L.M.; Liu, J.Y.; Qiao, M.; Xu, J.H. Isolation and biochemical characterization of two novel metagenome-derived esterases. *Appl. Biochem. Biotechnol.* **2013**, *169*, 15–28. [[CrossRef](#)] [[PubMed](#)]
206. Singh, D.N.; Gupta, A.; Singh, V.S.; Mishra, R.; Kateriya, S.; Tripathi, A.K. Identification and characterization of a novel phosphodiesterase from the metagenome of an Indian coalbed. *PLoS ONE* **2015**, *10*, e0118075. [[CrossRef](#)] [[PubMed](#)]
207. Yan, W.; Li, F.; Wang, L.; Zhu, Y.; Dong, Z.; Bai, L. Discovery and characterization of a novel lipase with transesterification activity from hot spring metagenomic library. *Biotechnol. Rep.* **2017**, *14*, 27–33. [[CrossRef](#)]
208. Kotik, M.; Vanacek, P.; Kunka, A.; Prokop, Z.; Damborsky, J. Metagenome-derived haloalkane dehalogenases with novel catalytic properties. *Appl. Microbiol. Biotechnol.* **2017**, *101*, 6385–6397. [[CrossRef](#)]
209. Apolinar, M.M.; Peña, Y.J.; Pérez-rueda, E.; Canto-canché, B.B.; Santos-briones, C.D.L.; Connor-sánchez, A.O. Identification and in silico characterization of two novel genes encoding peptidases S8 found by functional screening in a metagenomic library of Yucatán underground water. *Gene* **2016**, *593*, 154–161. [[CrossRef](#)]
210. Aviv-Reuven, S.; Rosenfeld, A. Publication patterns' changes due to the COVID-19 pandemic: A longitudinal and short-term scientometric analysis. *Scientometrics* **2021**, *126*, 6761–6784. [[CrossRef](#)]
211. Lai, O.M.; Lee, Y.Y.; Phuah, E.T.; Akoh, C.C. Lipase/esterase: Properties and industrial applications. In *Encyclopedia of Food Chemistry*; Melton, L., Shahidi, P.V.F., Eds.; Elsevier: Amsterdam, The Netherlands, 2018; pp. 158–167. ISBN 9780128140451.
212. Linares-Pasten, J.; Andersson, M.; Karlsson, E. Thermostable glycoside hydrolases in biorefinery technologies. *Curr. Biotechnol.* **2014**, *3*, 26–44. [[CrossRef](#)]
213. Liu, X.; Kokare, C. Microbial enzymes of use in industry. In *Biotechnology of Microbial Enzymes*; Brahmachari, G., Ed.; Elsevier: Amsterdam, The Netherlands, 2017; pp. 267–298.
214. Martínez, A.T.; Ruiz-Dueñas, F.J.; Camarero, S.; Serrano, A.; Linde, D.; Lund, H.; Vind, J.; Tovborg, M.; Herold-Majumdar, O.M.; Hofrichter, M.; et al. Oxidoreductases on their way to industrial biotransformations. *Biotechnol. Adv.* **2017**, *35*, 815–831. [[CrossRef](#)] [[PubMed](#)]
215. Alneyadi, A.H.; Rauf, M.A.; Ashraf, S.S. Oxidoreductases for the remediation of organic pollutants in water—A critical review. *Crit. Rev. Biotechnol.* **2018**, *38*, 971–988. [[CrossRef](#)] [[PubMed](#)]
216. Jatuwong, K.; Suwannarach, N.; Kumla, J.; Penkhrue, W.; Kakumyan, P.; Lumyong, S. Bioprocess for production, characteristics, and biotechnological applications of fungal phytases. *Front. Microbiol.* **2020**, *11*, 188. [[CrossRef](#)] [[PubMed](#)]
217. Berry, D.F.; Shang, C.; Zelazny, L.W. Measurement of phytase activity in soil using a chromophoric tethered phytic acid probe. *Soil Biol. Biochem.* **2009**, *41*, 192–200. [[CrossRef](#)]