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# Discovery of novel enzymes from extreme environments through metagenomic approaches: A mini-review

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## Abstract

Extremophilic microorganisms are able to survive under extreme conditions of temperature, pH, salinity and pressure, such as hypersaline soils, hydrothermal vents, acidic coal mines and soda lakes. These microorganisms have special genetic and physiological modifications to survive under abiotic and biotic stresses. Extremozymes are considered as an important source for different industrial and biotechnological applications. With the advent of meta-omics approaches, the discovery of novel enzymes has accelerated. Using function- and sequence-based metagenomics, a large number of enzymes have been identified and characterized directly from extreme environments. Many extremozymes such as proteases, amylases, esterases, cellulases, chitinases, oxidoreductases and hydrolases have special properties to work at a wide range of salt, pH and temperature. In this review, the discovery and industrial applications of novel enzymes from extreme environments through metagenomic approaches have been discussed. We have also explained the screening methods of novel extremozymes through function- and sequence-based metagenomics.

**Keywords:** Extremophiles; Industrial enzymes; Metagenomic approaches; Extreme environments

## 1. Introduction

Microorganisms are able to grow in different extreme environments including highly arid, saline, hot, cold, alkaline, acidic, high pressure and high radiations. According to natural environments, extremophiles are named according to their isolation source of extremity such as thermophiles (high temperature), halophiles (hypersaline), psychrophiles (low temperature), alkaliphiles (alkaline pH) and acidophiles (extremely acidic), piezophiles (high pressure) and UVR extremophiles (high level of UV radiation) [1-3]. Some microorganisms have the ability to survive under different extreme environments and they are called polyextremophiles, such as thermo-piezophilic microbes that can grow under high temperature and pressure, haloalkaliphilic microorganisms isolated from highly saline and alkaline environments [4-6].

Extremophilic microorganisms have great potential for different industrial and biotechnological applications. The extremozymes from these microorganisms have been used as biocatalysts for various industrial processes under extreme conditions [7]. These enzymes are more efficient for high temperatures and a wide range of pH as compared to mesophilic enzymes. Few decades ago, the discovery of extremophilic microorganisms was very limited and was based on cultivation methods. However, with the advancement in molecular ecology methods and sequencing techniques nowadays, the abundance of novel microorganisms representing a majority of previously unexplored and unculturable extremophiles has become relatively easier [8].

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Metagenomic techniques draw special attention to the emerging discipline of microbial ecology. These culture-independent approaches opened new horizons of undiscovered biochemical processes and biopolymers in nature [9]. Next generation sequencing technologies include 454 GS FLX/Roche, Illumina (Solexa) HiSeq and MiSeq, Ion torrent, Single-molecule real-time (SMRT) and Nanopore sequencing by Oxford Nanopore Technologies (MinION and PromethION) have usually been used for metagenomic studies. With the advent of next generation sequencing technologies, thousands of microorganisms can be identified in parallel. Due to genome-based techniques, even low abundant microorganisms can also be explored in this way [2]. With the help of advanced bioinformatics tools, microbial ecology can be studied comprehensively in different extreme environments [10].

The advancements in metagenomic approaches have elaborated our understandings about the complex nature of microbial diversity living in diverse and extreme environments. The continuous progress in sequencing technology made it easier to study the microbial population and its dynamics through sequence- and function-based metagenomics [2, 11]. Functional metagenomics and metatranscriptomics have accelerated the rate of gene discovery from various environmental samples. In sequence-based metagenomics, environmental DNA have been sequenced into short reads those ultimately assembled and different microorganisms, biomolecules and enzymes can be identified [12]. In functional metagenomics, different functional genes from environmental DNA can be cloned and expressed in culturable hosts especially *E.coli*. Coupling next generation sequencing with stable-DNA isotope probing, specific functional microbial communities can be targeted in the environments.

The main objective of this review is to discuss the different aspects of metagenomic analyses and applications of these approaches to understand the production of novel microbial enzymes and other biomolecules. We have also explained the diversity of extremophiles and their adaptations to survive under different extreme environments.

## 2. Extremozymes

Enzymes are used in various industrial, clinical and biotechnological applications including food, agriculture, textile, paper and pulp industry, biofuels and pharmaceuticals. They can be used as biocatalysts, analytical agents, therapeutic agents, and diagnostic tools [13, 14]. Most of the enzymes are specific in their reactions, faster, safer, and generate less bio-waste [15]. Enzymes usually work at a narrow range of temperature, pH, pressure, and organic solvents. In an enzyme's biochemical reaction, organic solvent usually increases the solubility of hydrophobic substrates and shifts the thermodynamic equilibrium from hydrolysis to condensation [14, 16].

### 2.1. Thermostable Enzymes

Thermophiles are microorganisms living in hot environments such as hydrothermal vents and hot springs. The microbial communities of hydrothermal vents comprise both types of prokaryotes i.e., archaea and bacteria found from sediments of hydrothermal vents, black smoker sulfides, and smoker fluids [17]. The discovered extremophiles from this ecology include Euryachaeotas, such as *Methanipyrun* and *Methanococcus* (methanogens), *Pyrococcus, Thermococcus* (thermophilic euryarchaeota), *Archaeoglobus* (sulfate and iron reducers) along with other nitrate reducers, sulfur reducers, and aerobes. Some hyperthermophiles, such as heterotrophs i.e., Crenarchaeota including *Desulfurococcus Hyperthermus,* and *Staphylothermus* were identified and characterized from these environments as shown in Fig. 1 and Table 1 [18-20].

Metagenomic analysis of the microbial communities in marine sediments from the Jan Mayen vent fields in the Norwegian-Greenland Sea and thermal pools in Kamchatka, Russia and Yellowstone hot spring, USA have been analyzed by using different approaches such as metagenomics and metatranscriptomics [21]. Functional metagenomics through sequence-based screening can be used for the discovery of thermozymes from different hyperthermophilic archaea and bacteria. A number of thermophilic enzymes, such as cellulases, amylases, chitinases, pectinases, lipases, proteases, laccases, etc. are preferably required for use in various industrial processes and biorefineries (Fig. 1 and Table 1) [22-24]. Functional metagenomic analysis of deep-sea hydrothermal vents showed that enzymes and exopolysaccharides (EPSs) from thermophilic bacteria, such as *Vibrio diabolicus* and *Geobacillus thermodenitrificans* could be used in regenerative medicines [25].

### 2.2. Psychrotolerant Enzymes

Psychrophiles or cold-adapted microorganisms are able to live in cold environments at temperatures less than 15 °C. Psychrophiles have anti-freezing proteins and enzymes that can work at low temperatures and help these microbes to live in cold environments. The exploration of Antarctic oligotrophic desert soil also showed the unique diversity, extremozymes and magnitude of microbial biomass beyond the predictions and expectations of the scientific

community [26]. Psychrophilic microorganisms can produce a number of novel enzymes with interesting applications in industrial production processes [27].

**Table 1** Industrially important extremozymes discovered through metagenomic approaches

| Extreme Environment | Enzyme | Geographical origin | Screening approach | Heterologous host | Reference |
|---|---|---|---|---|---|
| Hot | Glucosidase | Hydrothermal spring | Function-based | *E. coli* | [18] |
| | Lipase | Taptapani Hot Spring, India | Function-based | *E. coli* | [20] |
| | Esterases | Hot vent sediment, Vulcano Island | Function-based | *E. coli* | [19] |
| | Serine protease | Chumathang hotspring, India | Function-based | *E. coli* | [21] |
| | DNA polymerase | Yellowstone hot spring, USA | Sequence-based | *E. coli* | [22, 23] |
| | Amine transferase | Hot spring, Italy | Sequence-based | *E. coli* | [24] |
| Cold | Cellulases and Esterases | Miers Dry Valley, Antarctic soil | Sequence-based | *E.coli* | [25, 26] |
| | Carbohydrate degrading enzymes | Glacial ice of the Northern Schneeferner, Germany | Sequence-based | *E.coli* | [27] |
| | Esterases | Glacier soil, Arctic Pole | Function-based | *E. coli* | [28] |
| Acidic | NiFe–hydrogenase | *Tinto river (SW Spain)* | Function-based | *E. coli* | [34] |
| | Esterases | Acid Mines, Southeast China | Function-based | *E. coli* | [31] |
| | Amylase | Berkeley Pit Lake | Function-based | *E. coli* | [35] |
| Alkaline | Lipase | Marine sediments, South China Sea | Function-based | *E. coli* | [38] |
| | Amylase and protease | Alkaline Hot Springs, Madhya Pradesh, India | Sequence-based | *E.coli* | [37] |
| | α-amylases and β-galactosidases | Soda Lake near Wicklow, Ireland | Sequence-based | *E.coli* | [39] |
| Hypersaline | Esterase | Hypersaline Basins, Eastern Mediterranean Sea | Function-based | *E. coli* | [49] |
| | Na+/H+ antiporter protein | Brines Well Zigong, Sichuan (SW, China) | Sequence-based | *E. coli* | [42] |
| | Lipase | Great Salt Lake, Utah, USA | Function-based | Whole-metagenome sequence analysis | [48] |
| | Carbohydrate degrading enzymes | Brine soda lakes Kulunda Steppe (Altai, Russia) | Sequence-based | Whole-metagenome sequence analysis | [36] |

The enzymes from psychrophiles are especially important for industrial processes because no initial heating is not required, and they provide low energy consumption. Cold-active enzymes including cellulases, proteases, and esterases have different biotechnological and industrial applications such as the food and baking industry, paper and pulp industry and bioremediation processes [28]. Functional metagenomics of Antarctic soils described the identification of industrial enzymes including lipases, amylases, proteases and cellulases [27]. Microbial diversity showed the important bacterial diversity and functional metagenomics revealed the novel psychrophilic enzymes such as esterases that have special modifications to survive under cold conditions as shown in *Fig. 1 and Table 1 [29]*.

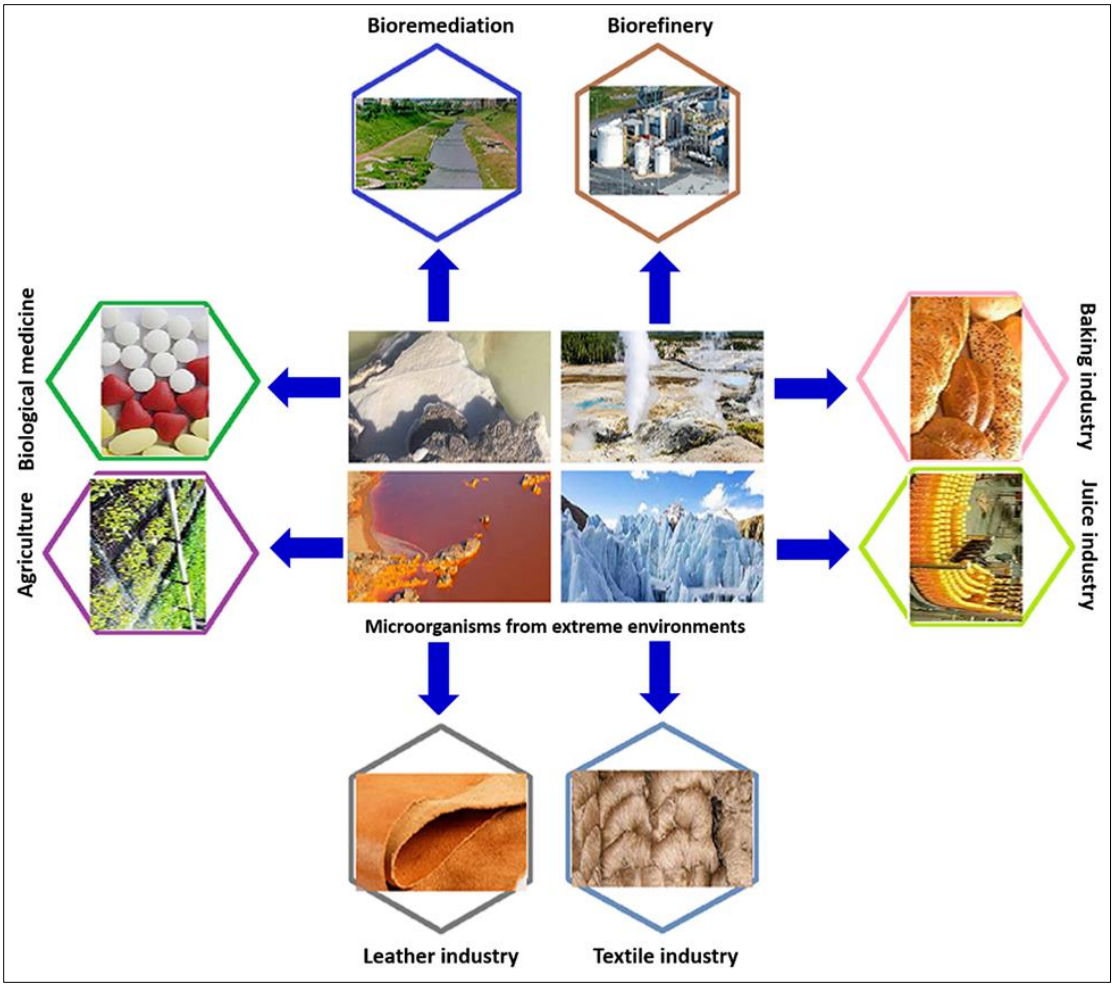


**Figure 1** Industrial and biotechnological applications of microbial enzymes identified from extremophiles

### 2.3. Acidophilic enzymes

Environmental factors such as salinity and pH affect the functional microbial communities living in extreme environments. Acidophiles have cytoplasmic pH 4-6 while alkaliphiles have internal pH 7.5-8.9 [30]. Acidophiles have been studied from a number of extreme environments, such as acid mine drainage, Solfatara (volcano) and acidic lakes [30, 31]. These environments usually have distinct microbial diversity as compared to other environments. Bacterial and archaeal genera, including *Acidithiobacillus, Sulfolobus, Ferrovum*, *Leptospirillum, Acidiphilium* and *Ferroplasma* were dominant at a low pH range from 2 to 4.3 [30, 32].

Acidophiles can survive at very low pH by using small organic molecules, such as proline, acetic acid, oxalic acid, and lactic acid to maintain their internal pH [30]. Some bacterial and archaeal strains can utilize sulfur compounds, such as sulfides, sulfates, or even elemental sulfur in their metabolism to survive under highly acidic environments [33]. Functional metagenomic analysis of acid mines showed that acidophilic enzymes including proteases, amylases, esterases, xylanases, α-glucosidases, sulfur dioxygenase, and iron-hydrogenases have distinct properties (*Fig. 1 and Table 1*). These enzymes have important biotechnological and industrial applications including in medicine, paper and pulp, leather, food, and textile industry [31, 34, 35].

### 2.4. Alkaliphilic enzymes

Alkaliphiles are microorganisms that are able to grow in alkaline environments with a pH above 9 but they usually show optimal growth at pH around 10. Alkaliphiles may coexist with neutrophiles under mild basic pH conditions. Alkaline environments, such as soda lakes, soda lake brines, carbonate springs, hyper-alkaline sediments, Dead Sea and alkaline/sodic desert environments represent the unique microbial diversity as compared to other ecosystems [5, 36, 37]. Metagenomic analysis of functional microbial communities from hyperalkaline environments such as marine sediments, alkaline hot springs, soda-lake near Wicklow, Ireland reported different proteins and extremozymes such as cellulase, lipase, protease, xylanase, glucosidase, chitinase, amylase, pectinase, and esterase that have the ability to work at extreme conditions of pH as shown in *Fig. 1 and Table 1* (7.5-10.37). Alkaliphilic enzymes have various industrial applications including paper and pulp industry, detergents, textile, medicine, and molecular biology [5, 38, 39].

### 2.5. Halotolerant Enzymes

Halophilic microorganisms are able to grow and survive under high salt conditions. Metagenomic analyses of hypersaline soils showed that increases in salinity cause changes in soil organic matter, loss of nutrients and changes in both abundance and composition of soil microbial communities [40, 41]. These microorganisms can survive against the harsh conditions of salinity, drought, high temperature, and low oxygen in their surroundings due to the formation of biofilms or osmoregulation by the accumulation of intracellular small organic molecules known as osmolytes [42-44].

Metagenomic studies of hypersaline showed that some halophilic bacterial genera including *Bacillus, Alkalimonas, Brachybacterium, Cronobacter, Halomonas, Halobacillus, Methylibium, Marinococcus, Oceanobacillus, Stenotrophomonas* and *Virgibacillus* and archaeal genera such as *Halobacterium, Halococcus* and *Haloferax* are sources of industrially related enzymes as shown in Fig. 1 and Table 1 [45-47]. Some studies on functional metagenomics and proteomics discovered a number of novel enzymes from halophilic bacteria and archaea from different hypersaline environments [36, 48, 42]. Ferrer et al. [49] reported that halophiles are good source of esterases from hypersaline Basins of Eastern Mediterranean Sea. A number of halophilic enzymes, such as cellulases, chitinases, proteases, amylases, pectinases, lipases and laccases, are preferably required for use in different industrial processes and biorefineries. The halophilic enzymes and proteins have special genetic modifications to work under high salt conditions (Fig. 1).

## 3. Identification of novel enzymes through metagenomic approaches

During the last two decades, the progress in culture-independent approaches based on the identification and characterization of microorganisms directly from environmental samples revealed the discovery of novel enzymes and other biomolecules from unculturable microorganisms. A number of studies on functional metagenomic analysis showed that amylases, lipases, cellulases, chitinases, proteases and DNA polymerases [9]. Using these approaches, a large number of industrially important biocatalysts were characterized as cellulases, chitinases, esterases, amylases and proteases as shown in *Fig. 2* [50]. Metagenomic analysis can be divided into two main categories including sequence-based and function-based or functional metagenomics which involves the expression of proteins or enzymes and their features, such as specific enzyme activity.

### 3.1. Sequence-based metagenomics

Sequence-based metagenomics including gene mining or PCR amplification of novel biocatalyst genes from environmental DNA can be used to identify different industrially important biocatalysts (*Fig. 2*). This technique is based on the amplification of enzyme encoded genes with primers designed specifically to the conserved regions or catalytic domains of enzymes [51]. Hydrolytic enzymes including proteases, amylases, cellulases, alcohol dehydrogenases and esterases have been identified by using this technique [39, 51, 52]. The main limitation of PCR based metagenomics is that only a few enzymes have been identified during the last two decades.

Recently, sequence-based metagenomics is considered a more attractive technique, as a large number of genes related to novel enzymes can be identified from single environmental DNA through high throughput sequencing. With the advancement of next generation sequencing (NGS) approaches, the discovery of enzymes has been promoted rapidly. On the basis of bioinformatic analyses, a large number of enzymes have been identified through in silico screening of environmental DNA from metagenomic libraries. In the last few years, cellulases, amylases, chitinases, proteases, esterases, oxireductases, nitrilases and many other enzymes have been discovered in extreme environments as shown in Table 1. Both high throughput sequencing and PCR detection of conserved regions of enzymes can be used for the discovery of novel enzymes as a new approach these days [36, 53].

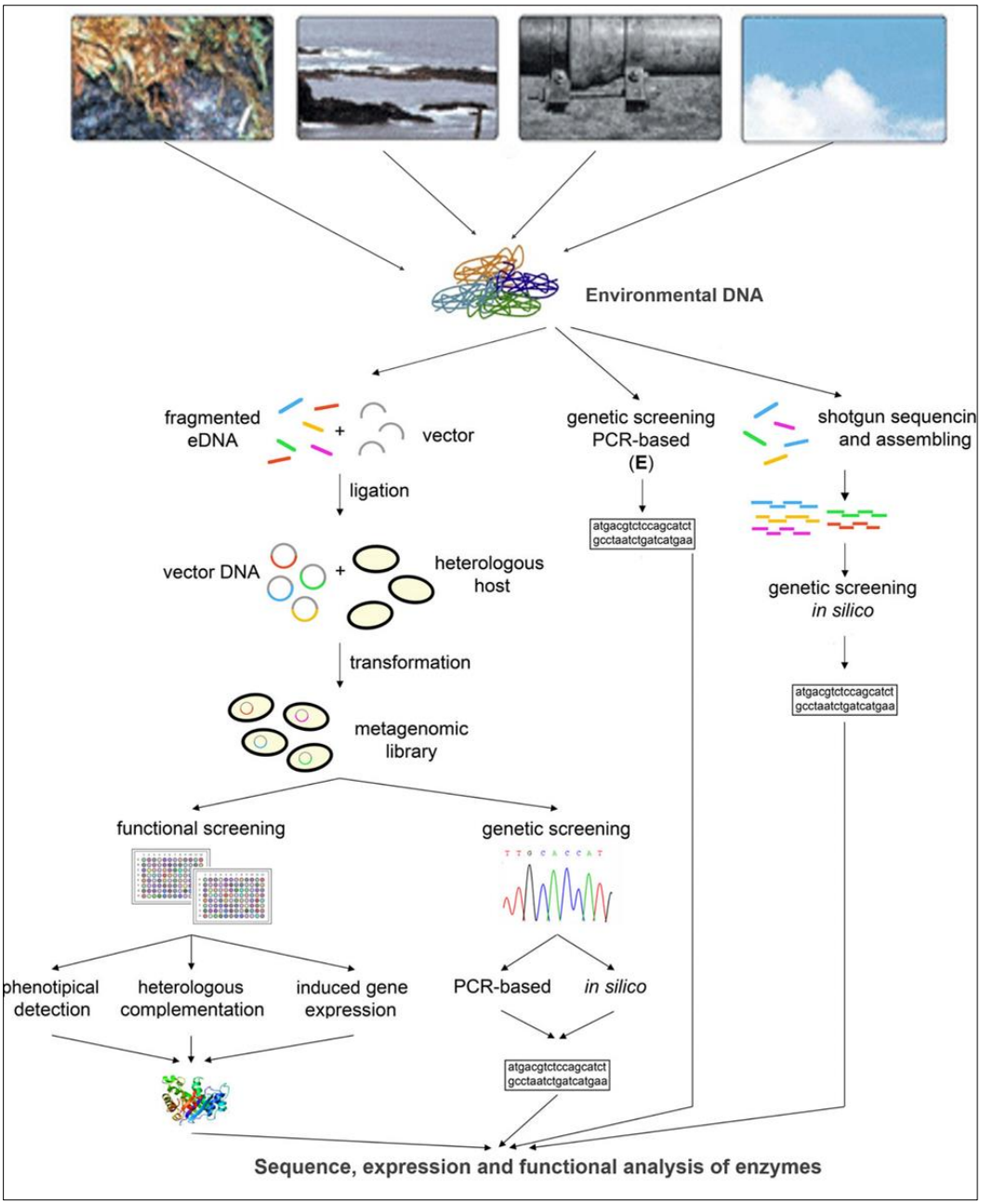


**Figure 2** Comparison of function- and sequence-based metagenomics for the discovery of novel extremozymes

### 3.2. Function-based metagenomics

In function-based metagenomic analysis, DNA fragments from environmental DNA are cloned into different hosts, e.g., E. coli and screened these clones on the basis of their protein expression. This approach leads to the discovery of new enzymes more efficiently [19, 20]. However, these techniques do not explain the regulation of these proteins and enzymes in the environment. Recently, metatranscriptomic analysis can be used to study the gene regulation and physiology of extreme microorganisms. Functional metagenomic and metatranscriptomic analyses can be utilized as a combined strategy to identify and characterize new enzymes from extremophilic microorganisms (Fig. 2 and Table 1). Some studies on functional microbial communities from extreme environments have used this approach. The metabolic profiles and gene expression of extreme microorganisms have been studied from acidic mines [53]. A variety of different enzymes including proteases, glycosyl hydrolases, amylases, esterases, cellulases, and chitinases have been identified by using function-based approaches [31, 35, 38]. The main challenge in function-based metagenomics is the requirement of specific hosts to create functional libraries and different screening methods.

## 4. Conclusion

With the advent of next generation sequencing techniques, functional microbial communities in extreme environments have been extensively studied during the last two decades. By using metagenomic analyses, about 95% unculturable microbial diversity from different environmental samples including soils, water, plant , and animal tissues. Technical challenges in case of function-based are the main limitation of hosts for heterologous expression of proteins and metagenome-derived enzymes. Sequence-based metagenomic analysis or whole metagenome analysis can provide a new dimension to identify and characterize novel proteins and enzymes. Usually, this technique is cost-efficient and produces a large amount of data that consequently leads to the discovery of new enzymes from extremophilic microorganisms. Currently, meta-omic approaches are considered the best way to discover and identify new enzymes or industrially important biocatalysts.

## Compliance with ethical standards

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## References

[1] Karan R, Singh SP, Kapoor S. A novel organic solvent tolerant protease from a newly isolated *Geomicrobium* sp. EMB2 (MTCC 10310): production optimization by response surface methodology. *Nat Biotechnol* 2011; 28: 136-145.

[2] Cowan DA, Ramond JB, Makhalanyane TP. Metagenomics of extreme environments. *Curr Opin Microbio.l* 2015; 25: 97-102.

[3] Jorquera MA, Graether SP, Maruyama F Bioprospecting and biotechnology of extremophiles. *Front Bioeng Biotechnol.* 2019; 7: 204.

[4] Patel RR, Patel DD, Thakor P, Patel B, Thakkar VR. Alleviation of salt stress in germination of *Vigna radiata* L. by two halotolerant *Bacilli* sp. isolated from saline habitats of Gujarat. *Plant Growth Regul.* 2015; 76(1): 51-60.

[5] Mukhtar S, Malik KA, Samina M Isolation, characterization of haloalkaliphilic bacteria isolated from the rhizosphere of *Dichanthium annulatum*. *J Adv Res Biotechnol* 2018a; 3:1-9.

[6] Atalah J, Cáceres-Moreno P, Espina G . Thermophiles and the applications of their enzymes as new biocatalysts. *Bioresour Technol* 2019; 280: 478-488.

[7] Mukhtar S, Mehnaz S, Mirza MS, Malik KA. Isolation and characterization of halophilic bacteria from the rhizosphere of halophytes and non-rhizospheric soil samples. *Braz J Microbiol.* 2019; 50: 85-97.

[8] Karan R, Grötzinger S, Vogler M. Single amplified genomes from Red Sea brine pool extremophiles-a source of novel enzymes. *Stud Univ Babes Bolyai Bio.l* 2019; 64: 76.

[9] Broaders E, O'Brien C, Cormac Gahan GM. Evidence for plasmid-mediated salt tolerance in the human gut microbiome and potential mechanisms. *FEMS Microbiol Ecol* 2016; 92: 1-8.

[10] Mukhtar S, Mirza BS, Mehnaz S. Impact of soil salinity on the structure and composition of rhizosphere microbiome. *World J Microbiol Biotech* 2018b; 34: 136.

[11] Gilbert JA, Jansson JK, Knight R The Earth Microbiome project: successes and aspirations. *BMC Biol* 2014; 12: 69.

[12] Hugenholtz P, Kyrpides NC A changing of the guard. *Environ Microbiol* 2009; 11: 551-553.

[13] Schäfer T et al. Industrial enzymes. In: Ulber R and Sell D, ed. White Biotechnology. Berlin: Springer, 2007: 59–131(2007).

[14] Illanes A, Cauerhff A, Wilson L. Recent trends in biocatalysis engineering. *Bioresour Technol* 2012; 115: 48–57.

[15] Anastas P,Eghbali N. Green chemistry: principles and practice. *Chem Soc Rev* 2010; 39: 301–312.

[16] Siddiqui KS, Parkin DM, Curmi PM. A novel approach for enhancing the catalytic efficiency of a protease at low temperature: Reduction in substrate inhibition by chemical modification. *Biotechnol Bioeng* 2009; 103: 676–686.

[17] Kashefi K, Lovley DR Extending the upper temperature limit for life. *Science* 2003; 301(5635): 934-934.

[18] Schröder C, Elleuche S, Blank S .Characterization of a heat-active archaeal β-glucosidase from a hydrothermal spring metagenome. *Enzyme Microb Technol* 2014; 57:48–54.

[19] Placido A, Hai T, Ferrer M. Diversity of hydrolases from hydrothermal vent sediments of the Levante Bay, Vulcano Island (Aeolian archipelago) identified by activity based metagenomics and biochemical characterization of new esterases and an arabinopyranosidase. *Appl Microbiol Biotechnol* 2015; 99: 10031–10046.

[20] Sahoo RK, Kumar M, Sukla LB. Bioprospecting hot spring metagenome: Lipase for the production of biodiesel. *Environ Sci Pollut Res* 2017; 24: 3802–3809.

[21] Wilkins LGE, Ettinger CL, Jospin G. Metagenome-assembled genomes provide new insight into the microbial diversity of two thermal pools in Kamchatka, Russia. *Sci Rep* 2019; 28: 3059.

[22] Singh R, Chopra C, Gupta VK. Purification and characterization of CHpro1, a thermotolerant, alkali-stable and oxidation-resisting protease of Chumathang hotspring. *Sci Bull* 2015; 60: 1252–1260.

[23] Moser MJ, DiFrancesco RA, Gowda K. Thermostable DNA polymerase from a viral metagenome is a potent RT-PCR enzyme. *PLoS ONE* 2012; 7:e38371.

[24] Ferrandi EE, Previdi A, Bassanini I. Novel thermostable amine transferases from hot spring metagenomes. *Appl Microbiol Biotechnol* 2017; 101: 4963–4979.

[25] Spanò A, Gugliandolo C, Lentinia V. A novel EPS-producing strain of *Bacillus licheniformis* isolated from a shallow vent off Panarea Island (Italy). *Curr Microbiol* 2013; 67: 21-29.

[26] Hu XP, Heath C, Taylor MP. A novel, extremely alkaliphilic and cold-active esterase from Antarctic desert soil. *Extremophiles* 2012; 16: 79-86.

[27] Simon C, Wiezer A, Strittmatter AW. Phylogenetic diversity and metabolic potential revealed in a glacier ice metagenome. *Appl Environ Microbiol* 2009; 75: 7519-7526.

[28] De Santi C, Altermark B, Pierechod MM. Characterization of a cold-active and salt tolerant esterase identified by functional screening of Arctic metagenomic libraries. *BMC Biochem* 2016a; 17:1.

[29] Møller AK, Søborg DA, Al-Soud WA. Bacterial community structure in High-Arctic snow and freshwater as revealed by pyrosequencing of 16S rRNA genes and cultivation. *Polar Res* 2013; 32: 17390.

[30] Sharma A, Kawarabayasi Y, Satyanarayana T Acidophilic bacteria and archaea: Acid stable biocatalysts and their potential applications. *Extremophiles* 2012; 16: 1-19.

[31] Kuang JL, Huang LN, Chen LX. Contemporary environmental variation determines microbial diversity patterns in acid mine drainage. *ISME J* 2013; 7: 1038-1050.

[32] Rousk J, Bååth E, Brookes PC. Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J* 2010; 4: 1340-1351.

[33] Chen L, Ren Y, Lin J. *Acidithiobacillus caldus* sulfur oxidation model based on transcriptome analysis between the wild type and sulfur oxygenase reductase defective mutant. *PLoS One* 2012; 7: e39470.

[34] Mirete S, de Figueras CG, Gonzalez-Pastor JE Novel nickel resistance genes from the rhizosphere metagenome of plants adapted to acid mine drainage. *Appl Environ Microbiol* 2007; 73: 6001-6011.

[35] Mirete S, Morgante V, Gonzalez-Pastor JE Functional metagenomics of extreme environments. *Curr Opin Biotechnol* 2016; 38: 143–149.

[36] Vavourakis CD, Ghai R, Rodriguez-Valera F. Metagenomic insights into the uncultured diversity and physiology of microbes in four hypersaline soda lake brines. *Front Microbiol* 2016; 7: 211.

[37] Choure K, Parsai S, Kotoky R. Comparative metagenomic analysis of two alkaline hot springs of Madhya Pradesh, India and deciphering the extremophiles for industrial enzymes. *Front Genet* 2021; 12: 643423.

[38] Peng Q, Wang X, Shang M. Isolation of a novel alkaline stable lipase from a metagenomic library and its specific application for milk fat flavor production. *Microb Cell Fact* 2014; 13: 1.

[39] Vester JK, Glaring MA, 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.

[40] Patel DD, Patel RR, Thakkar VR. Purification, characterization and application of lipoxygenase isoenzymes from *Lasiodiplodia theobromae*. *Appl Biochem Biotechnol* 2015; 175: 513-525.

[41] Mukhtar S, Ishaq A, Hassan S. Comparison of microbial communities associated with halophyte (*Salsola stocksii*) and non-halophyte (*Triticum aestivum*) using culture-independent approaches. *Pol J Microbiol* 2017; 66: 375-386.

[42] Xiang W, Zhang J, Li L. Screening a novel Na+/H+ antiporter gene from a metagenomic library of halophiles colonizing in the Dagong Ancient Brine Well in China. *FEMS Microbiol Lett* 2010; 306: 22-29.

[43] Borruso L, Bacci G, Mengoni A. Rhizosphere effect and salinity competing to shape microbial communities in *Phragmites australis* (Cav.) Trin. ex-Steud. *FEMS Microbiol Lett* 2014; 359: 193–200.

[44] Patel RR, Patel DD, Bhatt J, Thakor P, Triplett LR, Thakkar VR. Induction of pre-chorismate, jasmonate and salicylate pathways by *Burkholderia* sp. RR18 in peanut seedlings. *J Appl Microbiol* 2021; 131(3): 1417-30.

[45] Zhao S, Zhou N, Zhao ZY. Isolation of endophytic plant growth-promoting bacteria associated with the halophyte *Salicornia europaea* and evaluation of their promoting activity under salt stress. *Curr Microbiol* 2016; 73(4): 574-581.

[46] Mukhtar S, Mehnaz S, Malik KA Comparative study of the rhizosphere and root endosphere microbiomes of Cholistan Desert plants. *Front Microbiol* 2021; 12: 618742.

[47] Mukhtar S, Malik KA, Mehnaz S. Osmoadaptation in halophilic bacteria and archaea. *Res J Biotechnol* 2020; 15(5): 154-161.

[48] Spring S, Ludwig W, Marquez MC. *Halobacillus* gen. nov., with descriptions of *Halobacillus litoralis* sp. nov. and *Halobacillus trueperi* sp. nov., and transfer of *Sporosarcina halophila* to *Halobacillus halophilus* comb. nov. *Int J Syst Evo Microbiol* 1996; 46(2): 492-496.

[49] Ferrer M, Golyshina OV, Chernikova TN. Microbial enzymes mined from the Urania deep-sea hypersaline anoxic basin. *Chem Biol* 2005; 12: 895-904.

[50] Kellner H, Luis P, Portetelle D. Screening of a soil metatranscriptomic library by functional complementation of *Saccharomyces cerevisiae* mutants. *Microbiol Res* 2011; 166: 360–836.

[51] Coughlan LM, Cotter PD, Hill C. Biotechnological applications of functional metagenomics in the food and pharmaceutical industries. *Front Microbiol* 2015; 6: 672.

[52] Hjort K, Presti I, Elvang A. Bacterial chitinase with phytopathogen control capacity from suppressive soil revealed by functional metagenomics. *Appl Microbiol Biot* 2014; 98: 2819-28.

[53] Tripathy S, Padhi SK, Mohanty S. Analysis of the metatranscriptome of microbial communities of an alkaline hot sulfur spring revealed different gene encoding pathway enzymes associated with energy metabolism. *Extremophiles* 2016; 20: 525–536.