



REVIEW

# Metaproteomics: an emerging tool for the identification of proteins from extreme environments

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## Abstract

Microbial communities from extreme environments, such as saline, arid, hot, cold, acidic, or alkaline are especially important because they have special genetic and physiological modifications to function properly under extreme environments. They possess extremozymes and other biomolecules that can be used in various industrial processes, e.g., pharmaceuticals, paper manufacturing, degradation of complex organic molecules, biofuel production and food industries. With the advent of new sequencing technologies and ‘omics’ approaches, such as metagenomics, metatranscriptomics and metaproteomics, new windows have been opened to study the microbial ecology and functional microbial communities from extreme environments. Recently, metaproteomic analysis has been extensively used to explore the functional microbial communities from various extreme environments around the globe. In this review, we have focused on the microbial diversity analysis, identification of novel proteins, and enzymes from extreme environments, through metaproteomic approaches.

**Keywords** Functional microbial diversity · Extremozymes · Metaproteomics · Extreme environments

## Introduction

Extremophiles are a group of microorganisms having the capability of living in extreme environments. These are named according to their isolation source of extremity such as halophiles (hypersaline), thermophiles (high temperature), psychrophiles (low temperature), acidophiles (acidic pH) and alkalophiles (alkaline pH). These microorganisms have great potential for different biotechnological applications. They can be used for the production of novel enzymes and biopolymers (Borges et al. 2014; Boteva and Kamboorova 2018; Mukhtar et al. 2019a).

Different meta-omic approaches, such as metagenomics, meta-transcriptomics and meta-proteomics have been used to study microbial ecology as they allow deeper insights into the organismal and functional make-up of a natural environment. Meta-proteomics enable us to resolve the major catalytic units of microbial populations and helps to understand the genotype-phenotype linkages from in situ samples

(Wilmes et al. 2015). This technique is used as a tool for understanding the role of different members of a specific microbial community (Pieper et al. 2014). In the last decade, the metaproteomics approach has been used to study functional microbial communities from different environmental samples including ocean water, activated sludge, acid mine drainage biofilms, plant or animal tissues, etc. (Wilmes et al. 2015). Industrially important enzymes and other proteins from these microorganisms can be studied and utilized by using advanced omics-based approaches, such as functional metagenomics and metaproteomics (Kleiner 2019). Based on bacterial and fungal proteins and peptides, microbial diversity from various environments has been studied. Metaproteomic approaches can also be used for the restoration of contaminated and degraded soil by identification of different microbial proteins and enzymes with potential biotechnological applications (Bastida et al. 2009, 2015).

Different proteins and enzymes from the extreme environments can be identified based on different electrophoresis and mass spectrometry techniques and for final validation, various protein datasets are used. Samples for MALDI-TOF (matrix-assisted laser desorption/ionization) analysis are usually prepared by coating the protein sample with a matrix (Fig. 1). Upon ionization, proteins from a specific sample get protonated and separated on the basis of charge and mass

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ratio upon acceleration on fixed potential. These proteins are identified and measured using different mass analyzers (Sussulini and Becker 2011). LC-MS/MS (Liquid Chromatography with tandem mass spectrometry) is another useful technique to identify proteins from different environmental samples. This technique is based on the combined analysis of liquid chromatography and highly sensitive mass analysis capability of triple quadrupole mass spectrometry as shown in Fig. 1 (Everley et al. 2008).

Metaproteomics technique is still challenged due to limitations in protein extraction methods, computational analyses and available databases. For example, in case of soil metaproteomic analysis, the presence of humic acids, seasonal variability, and nestedness hinder the extraction of proteins with good quality Bunge 2016; Keiblinger and Riedel 2018; Mocali et al. 2010). Gans et al. (2005) reported the microbial diversity from soil based on metaproteomic analysis and it was estimated that about  $8 \times 10^6$  different taxa per gram of soil were present (Bastida et al. 2014). Nonetheless, this technique has been used for microbial diversity analysis and many recent studies have highlighted the need for better protein isolation methods from the environmental samples, especially soil (Mattarozzi et al. 2017; Mukhtar et al. 2018c; Nicora et al. 2013). A large number of proteins cannot be identified because of the absence of complete protein databases (Bastida et al. 2014; Keiblinger and Riedel 2018; Schneider et al. 2012).

There are several enzymes from different classes, present in soil that are involved in plant material decomposition. While the enzymes involved in litter decomposition can also provide insights to taxa involved in this process (Schneider et al. 2012). Plant-microbe interactions have been studied for many decades and now being analyzed through metaproteomics approaches. Bao et al. (2014) combined the spatial resolution of catalyzed reporter deposition-fluorescence *in situ* hybridization (CARD-FISH) and metaproteomics to study the methylocystaceae family of bacteria that inhibit the epidermal and vascular bundles cells of rice roots.

With relatively low cost of high throughput sequencing techniques, metagenomics and metatranscriptomics approaches are commonly used to study the functional microbial communities from various extreme environments. However, metaproteomics is still considered a complex technique and has been well-established in a few laboratories around the world (Wilmes et al. 2015). This review gives an overview of microbial diversity analysis from various extreme environments and explains the identification of novel proteins and enzymes from extreme environments through metaproteomic approaches.

## Metaproteomic analyses of extreme environments

### Saline environments

Halophiles live in a diverse range of habitats including salt mines, deep-sea brines, solar salterns, hydrothermal vents, marshy lagoons, hypersaline and alkaline lakes (Sarwar et al. 2015). Saline area in the world is increasing as a result of natural changes as well as anthropogenic effects on the environment (Mukhtar et al. 2018b; Oren 2002). Halophiles have the ability to survive under a wide range of salt concentrations because these microorganisms have developed special physiological and genetic modifications (DasSarma and DasSarma 2015; Mukhtar et al. 2019a, b).

Halophiles use two basic strategies to live in salinity affected environments (Karan et al. 2012; Mukhtar et al. 2019a). Halotolerant and halophilic bacteria, such as *Bacillus*, *Alkalimonas*, *Brachybacterium*, *Cronobacter*, *Halomonas*, *Halobacillus*, *Methylibium*, *Marinococcus*, *Oceanobacillus*, *Stenotrophomonas* and *Virgibacillus* use ‘compatible solute strategy’. They usually balance their osmotic pressure inside and outside of the cell by accumulation of osmolytes. They use small organic molecules, such as ectoine, betaine, trehalose, proline, glutamic acid, glutamine and other amino acids (Mukhtar et al. 2019b, 2020; Naghoni et al. 2017). Anaerobic halophilic bacteria and haloarchaea use ‘salt in strategy’ to balance their cytoplasmic salt concentrations. They use inorganic ions, such as potassium, sodium, magnesium and chloride.

A variety of proteins and enzymes including, amylase, protease, pullulanase, lipase, pectinase, xylanase and nuclease trehalose, proline, ectoine, sugars, polyols and proteins involved in signal transduction and stress responses are synthesized and used by halophilic bacteria and archaea to survive under hypersaline environments (Table 1) (Hanson et al. 2014; Mukhtar et al. 2019b, 2020; Pinar et al. 2014). Halophilic microorganisms use these molecules to protect their cells against desiccation, freezing or chemical denaturation (Delgado-García et al. 2014; Osman et al. 2019; Schneider et al. 2007). These osmolytes or halophilic enzymes stabilize the cellular membrane, reduce the freezing point of cytoplasm and maintain the internal osmotic balance under various extreme environments.

Halophilic enzymes and other proteins have also been used for bioremediation of polluted saline environments (Fig. 1) (Cowan et al. 2015; Lyszka et al. 2012). Halophilic microorganisms are considered as a rich source of therapeutic proteins and other compounds, such as antibiotics, anticancer proteins and important industrial enzymes (Morris et al. 2010; Mukhtar et al. 2019a; Shi et al. 2012). Halophilic proteins and other organic molecules have various

**Table 1** Identification of microbial enzymes and other biomolecules from various extreme environments using metaproteomic approaches

Extreme environment	Protein identification method	Biome/ Isolation source	Target enzymes/ other proteins	References
Saline	MALDI-TOF/TOF MS	Great Salt Lake, Utah	Lipase, amylase, protease, pullulanase, pectinase, xylanase, nucleases and proteins involved in signal transduction and stress response	Hanson et al. (2014)
	LC-MS/MS	Howz Soltan Lake (Iran)	Amylase, lipase, chitinase and protease and proteins involved in stress response	Morris et al. (2010)
	MALDI-TOF/TOF MS	Saltern crystallizer ponds (Spain)	Protease, amylase, chitinase, pectinases and nuclease	Fernández et al. (2014)
	LC-MS/MS	Marine solar saltern, Korea	Amylase, protease and DNase, chitinase and proteins involved in degradation of organic pollutants	Cowan et al. (2015)
	LC-MS/MS	Pink Salt Lakes in Camargue (France)	Protease, amylase and nuclease, xylanase, serine peptidase and proteins involved in signal transduction	Osman et al. (2019)
	MALDI-TOF/TOF MS LC-MS/MS	Hypersaline Lake Meyghan, Iran Himalatt salt lakes of the Algerian Sahara	Esterase, lipase and caseinase Esterase, xylanase, chitinase and inulinase	Naghoni et al. (2017) Boutaiba et al. (2011)
Arid	LC-MS/MS	Cold deserts	Lipases, esterase, cholesterol oxidase, ketoreductases, hydrolase and DNase	Ewing et al. (2015)
	MALDI-TOF/TOF MS	Arid soils	Esterase, lipase and amylase, xylanase and cryoprotective proteins	Bastida et al. (2015)
	LC-MS/MS	Desert soils	Esterase, protease, lipase, caseinase and proteins involved in signal transduction and stress response	Sánchez-Porro et al. (2007)
	LC-MS/MS	East Antarctica	Lipase, laccase, cellulase, chitinase, nuclease and esterase	Oren (2010)
	LC-MS/MS	Himalatt salt lakes of the Algerian Sahara	Amylase, cellulase and esterase	Boutaiba et al. (2011)
	Acidic	LC-MS/MS	Acid mines	Amylase, cellulase, sulfur dioxygenase oxidoreductase, xylanase, lipase, iron-hydrogenase, alcohol dehydrogenase and esterase
MALDI-TOF/TOF MS		Acid mines	Sulfur dioxygenase, galactosidase, serine peptidase, iron-hydrogenase, lipase and cytochrome oxidase	Zhang et al. (2016)
LC-MS/MS		Sulfide mines	Esterase, lipase, glucosidase, iron-hydrogenase and protease	Mueller et al. (2011)
LC-MS/MS		Acid mines	Esterase, glucosidase, amylase, iron-hydrogenase and oxidase, protease and lipase	Denef et al. (2009)

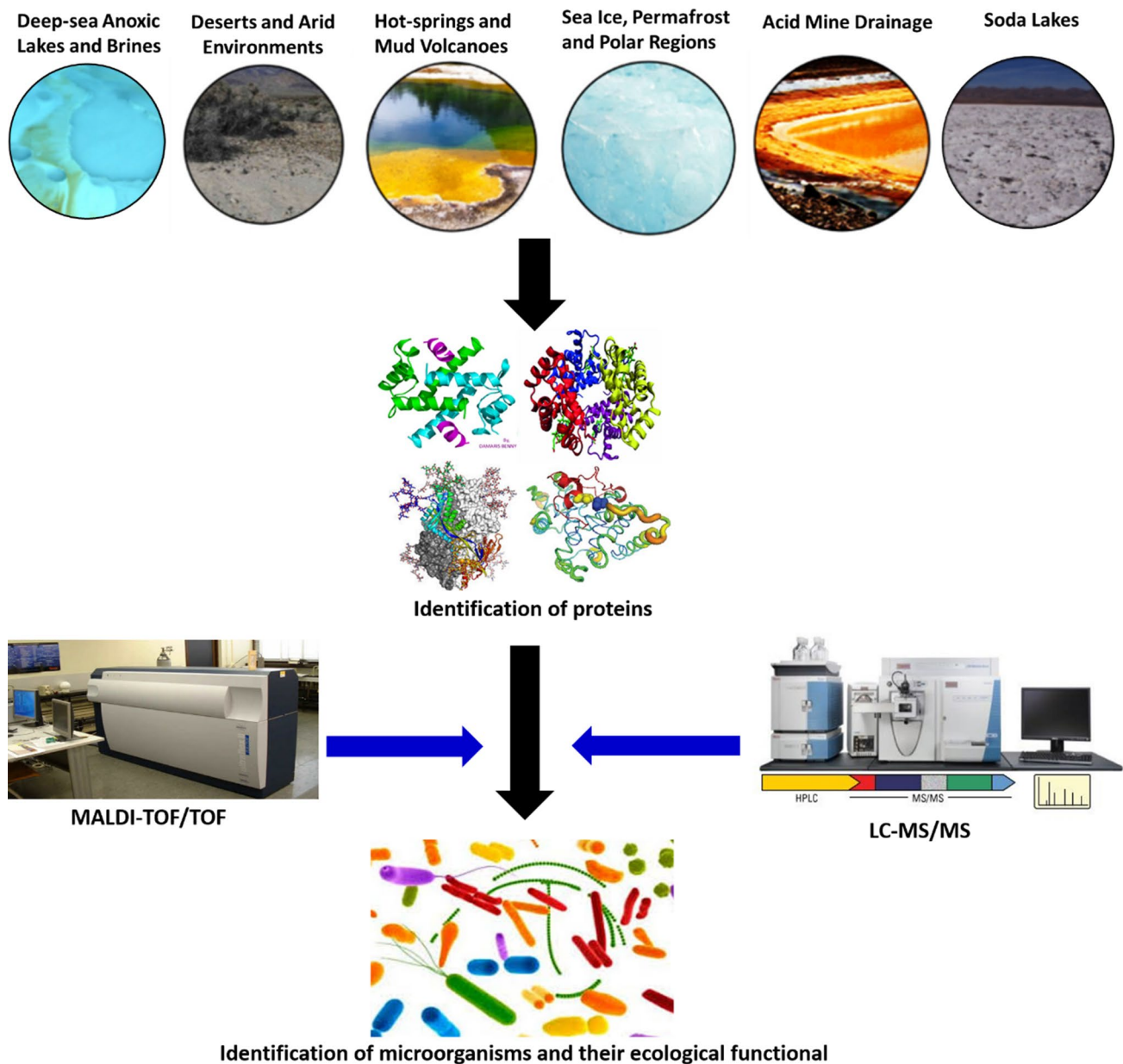
**Table 1** (continued)

Extreme environment	Protein identification method	Biome/ Isolation source	Target enzymes/ other proteins	References
Alkaline	LC–MS/MS	Soda lakes and halophyte rhizosphere	Esterase, lipase, chitinase, protease, iron-hydrogenase and amylase	Xiong et al. (2012)
	MALDI-TOF/TOF MS	Soda brine lakes	Pectinase, protease, iron-oxidase, cellulase, thermo-alkali-stable peptidase, alcohol dehydrogenase	Vavourakis et al. (2016)
	MALDI-TOF/TOF MS	Halophyte rhizosphere	Iron-oxidase, xylanase, amylase, protease and lipase	Preiss et al. (2015)
	LC–MS/MS	Soda lakes	Protease, iron-oxidase, cellulase, chitinase and lipases	Paul et al. (2016)
Hot	LC–MS/MS	Hot spring, Italy	Amylase, pullulanase, cellulase, chitinase, lipase, esterase, alcohol dehydrogenase and polymerases	Hensley et al. (2014); Martin et al. 2008
	MALDI-TOF/TOF MS	Deep-sea hydrothermal vent, USA	Amylase, lipase, esterase, cellulase, protease and polymerases and proteins involved in stress response and degradation of pollutants	López-López et al. (2013)
	LC–MS/MS	Hot springs, hydrothermal vents and volcanoes	Amylase, pullulanase, cellulase, protease, lipase, esterase, and hydrogenase	Kashefi and Lovley (2003)
	LC–MS/MS	Sea floor, hydrothermal vents and hot springs	Amylase, cellulase, lipase, xylanase, iron-hydrogenase, galactosidase and esterase	Schut and Adams (2009)
Cold	LC–MS/MS	Hot springs and oil wells	Cellulases, lipase, esterase, xylanase, thermo-alkali-stable peptidase and protease	Qi et al. (2017)
	LC–MS/MS	East Antarctica and Arctic polar sea ice	Lipase, cellulase and exopolysaccharides	Fang et al. (2010)
	LC–MS/MS	Arctic soils	Protease, laccase, and amylase	Bell et al. (2013)
	LC–MS/MS	South Coast of Korea	Amylase, chitinase, cellulase and esterase	Stokke et al. (2012)
	MALDI-TOF/TOF MS	Coastal sea ice and sediments	Esterase, lipase and amylase, cryo-protective exopolysaccharides	Qin et al. (2014)
MALDI-TOF/TOF MS	Antarctic soils	Amylase, cellulase, protease and antifreezing proteins	Williams et al. (2012)	
LC–MS/MS	Tundra soil	Protease, lipase, amylase and pectinase	Lauro et al. (2011)	

applications in food and nutraceutical industries, e.g., they are used for fermentation of fish sauces and production of carotenes (Boutaiba et al. 2011; Mukhtar et al. 2019a; Oren 2010). Osmoregulatory genes identified and characterized from halophilic microorganisms may be used for the development of transgenic crops with salinity tolerance (Mukhtar et al. 2019c). *Halobacterium* has the ability to produce a membrane protein, rhodopsin which absorbs sun light and can be used in memory and processing units of a computer.

Identification and characterization of stress related proteins and osmolytes including trehalose, glycine betaine,

proline dehydrogenase, ectoine, sugars, polyols can be used to study functional microbial diversity from various salinity affected environments. A number of proteins that are involved in signal transduction and metabolic pathways can be identified to study the interaction among different microorganisms (Cavicchioli et al. 2019; Talwar et al. 2020). Some recent studies on the characterization of transcriptionally active genes and their proteins through metatranscriptomic and metaproteomic analyses have provided useful information about the microbial communities that exist in extreme environments (Martinez et al. 2016; Overland et al. 2019).



**Fig. 1** Metaproteomics based approaches to identify microbial communities and their novel proteins and enzymes from various extreme environments

**Arid and semi-arid environments**

Water stress is one of the main limiting factors which affects plant growth and yield worldwide (Sharp et al. 2004). Globally, about 41.3% of Earth’s surface is affected by salt and drought, and this area continues to expand because of global climate change. Further increase in the percentage of abiotically stressed land will even more adversely impact the ability of the world’s population to grow enough food (Long and Ort 2010). Drought stress leads to cellular dehydration, which ultimately causes osmotic stress, thus hampering

cell expansion (Bartels and Sunkar 2005). Stomata remain closed due to deficiency of water and thus reduce the rate of photosynthesis and plant growth (Chen and Murata 2008; Yang et al. 2009).

Metaproteomic and metagenomic analyses of different arid and semi-arid environments showed that bacterial and archaeal genera including *Pseudomonas*, *Chromohalobacter*, *Rhodococcus*, *Actinopolyspora*, *Marinomonas*, *Halobacterium* and *Halococcus* were found to be dominant. A number of proteins and enzymes including proteases, cellulases, amylases, chitinases, oxidases, alpha hydrolase,



pyrroline-5-carboxylase, exo-polyphosphatase, universal stress protein UspA, and biopolymers, such as polyhydroxy alkanooates, exopolysaccharides, carotenoid pigments, osmolytes and bacteriorhodopsin were identified from these environments and ultimately used to analyse the microbial diversity (Chiang et al. 2019; Ewing et al. 2015; Roca et al. 2013; Talwar et al. 2020). Drought also affects growth of bacteria and archaea by physiological and genetic modifications in proteins' structure, and causes a change in activity, assembly and folding of different proteins (Chaplin 2006; Julca et al. 2012; Manzanera et al. 2002).

Drought tolerant microorganisms utilize osmolytes, such as proline, betaine, ectoine and trehalose to protect their cells under water stress environments (Julca et al. 2012; Mukhtar et al. 2019c; Sánchez-Porro et al. 2007). The drought-tolerant rhizobacteria have the ability to thrive in such extreme conditions by using mitigation strategies including, nitrogen fixation, phytohormone production, minerals solubilization (P, K, Zn), ACC deaminase (1-aminocyclopropane-1-carboxylate) and siderophores production. These bacteria produce several antifungal and antibacterial compounds to induce systemic resistance in the developing plant roots (Glick et al. 1999; Conrath 2006; Kosova et al. 2015; Yang et al. 2009). The number of potential targeted enzymes were xylanases, proteases, cellulases, amylases, and biopolymers, such as polyhydroxy alkanooates, exopolysaccharides, carotenoid pigments, osmolytes and bacteriorhodopsin that can be used for different biotechnological applications (Table 1). Some plant proteins, such as sigma factor RpoH1 induce the production of ACC deaminase and phytohormones in rhizobacteria to enhance plant growth under abiotic stress (Defez et al. 2016; Ewing et al. 2015).

### Acidic environments

Acidic environments, such as acid mine drainages and marine volcanic vents are present around the world. Microorganisms that grow in acidic environments have the specialized molecular mechanisms which enable them to survive in such harsh conditions (Johnson and Hallberg 2005; Xie et al. 2011). In acidic conditions, protons enter in a cell to reduce the cytoplasmic pH (Richard and Foster 2004; Zhang et al. 2016). Acidic pH may lead to uncoupling of oxidative phosphorylation and unfolding of proteins (Denef et al. 2009; Richard and Foster 2004). This may also cause damage to many cellular structures as well as disruption of many biological processes in the cell and may lead to cell death (Small et al. 1994). For instance, amino acid decarboxylase systems are expressed by *E. coli* in which a reductive decarboxylation of the substrate that is usually glutamate, arginine or lysine, consumes a proton and ultimately results in decrease of free proton concentration in cytoplasm (Foster 2004).

Microbial diversity analysis through metaproteomic approaches showed that acidophiles including *Acidithiobacillus*, *Acidianus*, *Leptospirillum*, *Acidiphilium* and *Ferroplasma* have several protective proteins to survive under acidic stress environments (Table 1). A number of studies on metaproteomic analysis from different acid mines across the globe reported more than 2500 proteins. These proteins may be involved in the various microbial functions including metabolism, cell signaling, defense mechanisms, abiotic and biotic stresses (Wilmes and Bond 2004). Identification and characterization of these proteins help to study the complex microbial communities associated with acidic regions (Simon et al. 2009).

Some of these are DnaK and GroEL chaperone machines and DNA repair enzymes (Thompson and Blaser 1995). HdeA and HdeB are periplasmic acid chaperones that prevent the aggregation of periplasmic proteins in bacteria during acidic stress (Dahl et al. 2015). Several industrially important enzymes such as proteases, amylases, cellulases, chitinases and lipases have been identified and characterized from the acidic environments (Mueller et al. 2011; Qi et al. 2017; Tang et al. 2014) through metaproteomics analysis. Most frequently identified proteins belonged to *Lactobacillus* spp., while the proteins from *Clostridium* spp. and *Streptococcus* spp. were also identified. The most frequently identified enzymes were pyruvate kinases and heat shock chaperones (Fig. 1).

### Alkaline environments

Microorganisms that can grow at alkaline pH range are known as alkaliphiles (Kevbrin 2019). Alkaliphilic microorganisms grow above pH 8, usually at pH 9–12 (Horikoshi and Bull 2011; Horikoshi et al. 2011). These microorganisms are omnipresent and have been studied from different extreme environments. Alkalinity in an environment can be caused by ecological processes or may be due to human activities. It might be possible that neutralophilic microorganisms are responsible for the development of alkaline conditions (Kevbrin 2019). Alkaliphiles can be found in soda lakes, the sites of serpentinization, ocean, soils, man-made alkaline sites, microbially mediated alkalization, and alkaliphilic eukaryotes (Kevbrin 2019). The soda lakes are widely spread around tropical, subtropical and intracontinental cryo-arid zones of the Earth (Deocampo and Renaut 2016; Vavourakis et al. 2016; Xiong et al. 2012).

Alkaliphiles have developed an adaptive mechanism for their survival by producing extracellular alkaline tolerant enzymes, such as cellulase, amylase, lipase, protease, xylanase, glucosidase, esterase and chitinase which are stable and functional at high alkaline conditions (Fujinami and Fujisawa 2010; Khalikova et al. 2019). Alkaliphiles produce several organic acids, such as acetic acid, lactic acid,

formic acid and malic acid which play an important role in an array of industrial processes (Kulshreshtha et al. 2012). Some alkaliphiles produce siderophores and carotenoids that have many applications (Mamo and Mattiasson 2016; Preiss et al. 2015).

Sukul et al. (2018) reported the identification of a novel gene that encodes a lipolytic enzyme, labeled as ML-005 by using functional metaproteomics techniques. This protein was expressed heterologously in *E. coli* and characterized biochemically. ML-005 shows lipolytic activity to short chained substrates with the preferred substrate being p-nitrophenyl-butyrate, which makes ML-005 an esterase (Table 1). Through homology analysis and site directed mutagenesis, Ser-99, Asp-164, and His-191 are identified as a catalytic trio of enzymes. The optimal pH and temperature were 8 and 45 °C, respectively. It showed the tolerance of a wide range of pH (5–12), temperature, and salt concentration.

Some recent studies on metagenomics and metaproteomics from oil contaminated alkaline soils described the identification of novel proteins and enzymes (Sukul et al. 2018). Microbial diversity analysis of alkaline environments showed that bacterial and archaeal genera, such as *Bacillus*, *Thioalkalivibrio*, *Serpentinomonas*, *Chromobacterium*, *Exiguobacterium*, *Halobacterium* and *Halalkalicoccus* were dominant. A number of metaproteomic studies have reported that extremozymes, such as protease, xylanase, cellulase, lipase, amylase, esterase, glucosidase, chitinase and pectinase from alkaliphilic microorganisms have the ability to work at extreme pH (7.5–10.37) (Mirete et al. 2016; Vavourakis et al. 2016; Mukhtar et al. 2018a).

### Hot environments

High temperature induces heat shock response in bacteria which helps them to survive in such extreme conditions (Lüders et al. 2009). Thermophiles are classified as moderate thermophiles (50–60 °C), extreme thermophiles (60–80 °C), and hyperthermophiles (80–110 °C), based on their growth temperature (Gupta et al. 2014; Khalil 2011). Among all extremophiles, thermophilic microorganisms, such as *Thermococcus*, *Pyrococcus*, *Pyrobaculum*, *Thermotoga*, *Alteromonas* and *Geobacillus* are the most popular microorganisms in many biotechnological processes (Mohammad et al. 2017; Schut and Adams 2009; Singh et al. 2011).

Heat shock proteins are playing a vital role in various scientific and industrial applications, for instance, the process in which heterologous proteins' production is induced through elevated temperature (Hensley et al. 2014; Han et al. 2004; Kashefi and Lovley 2003; Lüders et al. 2009). Chaperons and proteases are the most common heat shock proteins which stimulate protein folding, refolding, quality control and protein breakdown (Lüders et al. 2009). In recent decades, the thermostability of extreme thermophiles attracts

molecular biologists and biotechnologists to use these microorganisms on the platform of metabolic engineering by developing extraordinary molecular genetic tools. Now, biofuel and chemical manufacturing at high temperatures is due to recombinant extreme thermophiles (Liu et al. 2015; Zeldes et al. 2015).

During recent years, metaproteomics has been 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 different industrial processes and biorefineries (Keiblinger et al. 2012; Liu et al. 2015; López-López et al. 2013; Williams et al. 2012). These thermostable enzymes have specific features which help thermophiles to survive at extreme temperature as well as make them stable against a range of alkaline and acidic pH, solvents and detergents (Table 1) (Bhalla et al. 2013; Dettmer et al. 2013; Martin et al. 2008; Mohammad et al. 2017).

Microbial diversity from the deep sea hydrothermal vents and hot springs has the ability to adapt to extreme environment through the expression of certain transporter proteins such as ATP binding cassette (ABC)-type, glycine betaine transporter, cell signaling proteins, dehydrogenases, hydrogenases and proteins involved in DNA processing, nucleic acid binding and refolding. Expression level of these proteins from marine environments can be used to identify microorganisms such as *Pelagibacter*, *Rhodobacter* and *Prochlorococcus* and their role in nitrogen and carbon cycling in these environments (Azam and Malfatti 2007; Hanson et al. 2014). Metaproteomics and functional metagenomic analysis of deep-sea hydrothermal vents showed that specific proteins, enzymes and exo-polysaccharides (EPSs) from thermophilic bacteria, such as *Alteromonas infernus*, *Geobacillus thermodenitrificans* and *Vibrio diabolicus* can be used in various biotechnological applications including industrial processes and regenerative medicines (Arena et al. 2009; Spanò et al. 2013).

### Cold environments

Cold environments are predominant over the earth and the microorganisms inhabiting such environments are called psychrophiles (Morita 1975). Mechanisms that enable microorganisms to survive in cold adaptation involve the expression of cold shock proteins and structural adjustment of enzymes (Table 1). Other mechanisms include maintenance of membrane fluidity, and translation and transcription machinery adaptation (Barria et al. 2013). Reactive oxygen species (ROS) which cause oxidative stress and level of oxygen solubility at low temperatures (also generate the ROS) may affect tricarboxylic acid cycle (TCA), glycolysis, electron transport chain and pentose phosphate pathway

(Piette et al. 2012). These underlying mechanisms are not fully understood which help the bacteria to adapt the cold environments (Bell et al. 2013; Fang et al. 2010; Myka et al. 2017).

The results of six functional metagenomic datasets obtained from Antarctic Lake Joyce were used to get information about cold adaptation proteins. Other proteins observed were ice nucleation protein, antifreeze proteins, trehalose synthase, fatty acid desaturase and cold-shock DEAD-box protein A. A cold-shock family of proteins called CSPs, was also reported that included CspA, CspB, CspD, CspC, CspG and CspE (Liljeqvist et al. 2015).

Some cold-tolerant bacterial genera including *Psychromonas*, *Photobacterium*, *Arthrobacter*, *Zunongwangia Micrococcus*, *Pseudomonas* and *Marinomonas* have the ability to produce antifreeze proteins (Table 1). These proteins can be used in microbial fermentations at low temperatures (Nunn et al. 2015; Simon et al. 2009; Stokke et al. 2012). The psychrophilic microorganisms can produce a number of novel enzymes with applications in industrial processes (Bell et al. 2013; Qin et al. 2014; Williams et al. 2012). Several bacteria can synthesize the polyhydroxy, alkanoyates (PHA) to respond to cold environments. These are reverse polymers and have important physiological roles. The proteomic analysis of these bacteria showed an increase in PHA depolymerase at a lower temperature (around  $-10^{\circ}\text{C}$ ). This depicts the PHA utilization at low temperatures.

To survive under cold conditions, microorganisms also use the compatible solutes, e.g., glycine, betaine, trehalose, sorbitol, glycerol, sucrose, mannitol and ectoine that play an important role in osmoregulation as well as in cryoprotection. These molecules can scavenge the free radical, reduce the cytoplasm freezing point and stabilize cellular membrane under cold conditions (Collins and Deming 2013; Lauro et al. 2011). Ghobakhlou et al. (2015) also reported an increase in the level of threonine, valine and sarcosine in Arctic isolate *Mesorhizobium* sp. strain N33 when grown at  $4^{\circ}\text{C}$ . The presence of an envelope was observed in both, Gram-negative and Gram-positive, bacteria to avoid stiffness at low temperatures to maintain membrane fluidity (Médigue et al. 2005; Rodrigues et al. 2008).

Functional characterization of microbial communities from cold environments showed that several essential enzymes and proteins involved in energy production and metabolism, ABC transporters, proteins required for stress adaptation were dominantly reported. The identification of these proteins revealed that bacterial genera including *Halomonas*, *Pseudomonas*, *Marinobacter*, *Bacillus*, *Arcobacter* and *Desulfobacter* were more abundant as compared to others (Cavicchioli et al. 2019; Collins and Deming 2013).

## Future prospective

Extremophiles are a sustainable source and can be used for the development of bio-based economy. One of the main challenges in metaproteomic analyses includes genetic heterogeneity within the microbial communities, uneven distributions of species and changes observed in protein expression levels in different microorganisms. Identification and characterization of proteins involved in various metabolic activities and adaptation to abiotic and biotic stresses by using metaproteomic approaches can be helpful to study specific microbial communities or individual microorganisms from extreme environments that cannot be isolated or cultivated in a laboratory. Another exciting new direction is that metagenomic and metatranscriptomic approaches should also be studied parallel to check cross-contamination or other errors that may affect the homologous protein(s) identification (Vilanova and Porcar 2016; Wang et al. 2020). By using more efficient methods for proteins' extraction from various environmental samples and mass spectrometry analyses, the full potential of metaproteomic approaches can be studied. Metaproteomic analyses can be improved by using advanced software tools with the capability of handling large datasets and they should be user friendly. In future, the cost for metaproteomic analysis should be reduced as in the case of DNA sequencing, so that this technique can be used to study proteins and their applications from various unexplored environments (Chiapello et al. 2020; Deutsch et al. 2020). Despite all hurdles, metaproteomic approaches can also be used to analyze and develop a link between microbial diversity and functions of microbial communities and ultimately help in studying ecological changes.

## Conclusions

Advancement in meta-omics approaches, such as metagenomics, metatranscriptomics and metaproteomics has been used to uncover the complex composition as well as the key functional traits responsible for the survival of microorganisms from extreme environments. We can say that metaproteomics came as a boom in microbiology to study functional microbial communities from extreme environments including hypersaline, sodic lakes, deep sea hydrothermal vents and frozen lakes of polar regions. Metaproteomic approaches enhance the understanding of the functional microbial communities from various extreme environments and they can be used to discover novel genes, enzymes and other proteins with great biotechnological potential.



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