



METAPROTEOMICS APPROACH FOR PROTEIN ANALYSIS OF HALOTOLERANT

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ABSTRACT

Halophiles have evolved two basic strategies to survive in salt stress conditions. In 'compatible solute' strategy, most of the halophilic bacteria cope with their external environments by accumulating small, highly water soluble organic compounds like glycine betaine, proline, glutamine, potassium, glutamic acid and ectoine. In 'salt in' strategy, bacterial and archaeal cells maintain internal environment by accumulating high concentrations of potassium chloride. Accumulation of different compatible solutes can stimulate the bacterial growth in salt stress conditions. Halophilic and halotolerant bacteria can also be used to degrade different organic compounds in bioremediation process.

KEYWORDS: halophilic, betaine, proline, archaeal.

INTRODUCTION

The microbial diversity of extreme environments is of great interest and use. Examples of these extreme environments are like arid regions, thermophilic, saline, alkaline and acidic environments. Microbial diversity residing here is important because these microorganisms have developed special mechanisms that make them to survive in highly saline environments (Bastida et al. 2013). Halophilic microorganisms are salt-loving organisms that live in saline environments. Halophilic microorganisms include a variety of heterotrophic, phototrophic and lithotrophic bacteria (*Bacillus halodurans*, *Halomonas*, *Nesterenkonia* and *Chromohalobacter*) and heterotrophic and methanogenic archaea (*Halobacterium* sp., and *Natrococcus* sp.) (Oren, 2002). Halophiles have developed various mechanisms to cope with external salt stress environment (Karan et al., 2012). Halophiles have evolved two basic strategies to survive in salt stress conditions. In 'compatible solute' strategy, most of the halophilic bacteria cope with their external environments by accumulating small, highly water soluble organic compounds like glycine betaine, proline, glutamine, potassium, glutamic acid and ectoine. In 'salt in' strategy, bacterial and archaeal cells maintain internal environment by accumulating high concentrations of potassium chloride. Accumulation of different compatible solutes can stimulate the bacterial growth in salt stress conditions. Previous studies on osmoregulation in bacteria have shown that different transporters, channel proteins and enzymes can mediate solute accumulation and release (Pinar et al., 2014).

Halophilic bacteria and archaea can grow in hypersaline environments. These microorganisms have special physiological and genetic characteristics to survive under salt stress conditions. Halophilic bacteria have adapted different mechanisms to cope with saline conditions. Mostly bacteria have ability to accumulate small, organic molecules (compatible solutes) like glycine betaine, ectoine, glutamate and proline. Different enzymes and other proteins in halophilic bacteria play an important role in bacterial flexibility and adaptation. Halophilic bacteria have also been recognized as agents of spoilage of meat and fish. Some halophilic bacteria have been used for fermentation of protein-rich foods. Metaproteomics is the large-scale characterization of entire protein complement of environmental samples at a specific time. Among the different 'omics' approaches, metaproteomics is used to study microbial ecology now. It provides deeper insight into the structure and functional of microbial communities.

Different classes of chemical compounds, cyclic amino acids, enzymes, betains, sugars and polyols have been involved in salt tolerance. These compounds also protect biomolecules and whole cells against denaturation caused by deciccation, freezing, heating or chemical agents (Schneider et al. 2007). Halophiles have novel enzymes with inherent ability to function under salt stress conditions (Delgado-García et al., 2014). Certain enzymes produced by halophiles are considered useful for bioremediation of pollutants in saline habitats (Dastgheib et al., 2011) and production of important biomolecules, i.e., exopolysaccharides and phytohormones (Liszka et al., 2012). Halophiles have

been known as a rich source of novel enzymes, therapeutic compounds like antibiotics, immunosuppressive proteins and anticancer agents (Shi et al., 2012).

Different 'meta-omic' approaches like metagenomics, metatranscriptomics and metaproteomics have been used to study microbial ecology as they allow deeper insights into organismal and functional make-up of a natural environment (Wilmes et al., 2015). Microbial communities from different environments (soil, sea water and fresh water, activated sludge, human gut microbiota) have been investigated by using metaproteomic studies. There are virtually no studies on the metaproteomics of the rhizospheric soil halophytes. Thus, objective of the present study to identify and characterize different proteins involved in osmoregulation of halotolerant and halophilic bacteria through metaproteomic analysis. This information will allow us to better understand the mechanisms which enable the plants to survive under salt stress conditions.

Molecular evidence has greatly increased the importance of microbial diversity especially in extreme environments. Halophiles are salt loving organisms that flourish in saline environments. Halophiles are characterized on the basis of salt requirement for their growth in saline environments while halotolerant microorganisms do not salt for their growth although they can grow in hypersaline environments (Amoozegar et al., 2016). Halophiles can be classified into three groups on the basis of salt requirement for their growth: slight halophiles grow optimally at 0.2-0.85M NaCl concentration; moderate halophiles can grow at 0.85-3.4M NaCl concentration and extreme halophiles can grow optimally at 3.4-5.1M NaCl concentration. Many halophilic and halotolerant bacteria can grow at a wide range of salt concentrations dependent on environmental and nutritional factors.

The domain bacteria included both halophilic and halotolerant bacteria which spread over large number of phylogenetic groups. These bacteria included members of Proteobacteria (*Halomonas*, *Chromohalobacter*), Firmicutes (*Halobacillus* and *Virgibacillus*), Actinomycetes (*Nocardia* and *Nesterenkonina*) and Bacteroidetes (*Flavobacterium*). Most of the halophiles under domain bacteria are moderate halophiles and can grow under wide range of salt concentrations (DasSarma et al., 2015). Halobacteriaceae is the largest family of haloarchaea. It comprises 36 genera with 129 species (*Halobacterium* sp., *Halococcus* sp. and *Natrococcus* sp.). They can survive at high salt concentrations up to 5M NaCl (Oren, 2012). Previous studies on solar salterns showed the occurrence of a major new phylotype, called nanohaloarchaea with small cell size (50 μ m) (Narasingarao et al., 2011). The first haloarchaeal genome completed was for *Halobacterium* sp. NRC-1, which has a 2.5 Mbp genome (DasSarma et al., 2006).

Halophiles live in diverse range of habitats including salt mines, deep sea brines, solar salterns, hydrothermal, marshy lagoons, hypersaline and alkaline lakes (Sarwar et al., 2015). The diverse range of halophiles has allowed these microbes to develop rare skills for their survival in nature. Saline area in the world increases as a result of global natural changes as well as anthropogenic impacts on the environment (Oren, 2002). Thalassohaline environments were formed by the evaporation of sea water leading to the increase in sodium and chloride ions with alkaline pH. In Pakistan, Khewra Salt Mines also provides a rich and extensive habitat for halophytes and halophile microorganisms. Rhizosphere microbiome of halophytes contains of a wide diversity of halotolerant and halophilic microorganisms (Mukhter et al., 2016).

Halophiles maintain their internal ion concentration or osmotic balance by accumulating salts internally to survive under extreme conditions (Achtman and Wagner, 2008). Different halophiles have evolved various mechanisms to survive in extremely saline environments. Most of the halophiles use the compatible solute strategy and Na⁺/H⁺ antiporter system based sodium pumps for the maintenance of internal osmotic environment (Paster et al., 2013). Compatible solutes include organic compounds like glycine betaine, proline, glutamine, potassium, glutamic acid and ectoine. Mostly bacteria use two major groups of compatible solutes including polyols and sugars and amino acids derivatives to maintain their osmotic balance (Roberts, 2000; Riedel, 2012). Halophilic archaea and anaerobic bacteria use salt in strategy to survive under hypersaline conditions. They maintain their internal environment by accumulation high concentration of KCl ions. Haloarchaea are mostly amino acid utilizing facultative aerobes which require a number of growth factors and slightly elevated temperatures (35-45°C) for optimal growth. Members of the Halobacteriaceae have some distinctive features such as gas vesicles, purple membrane and red-orange carotenoids (DasSarma et al., 2010b).

Halophilic bacteria and archaea both have great biotechnological potential. Halophilic bacteria can be used in food (fermentation of soy and fish sauces) and nutraceutical industries (β -carotene production) (Oren, 2010). Salt tolerant genes from halophiles can be used to develop transgenic crops with salt tolerance. *Halobacterium* produces an integral membrane protein, bacteriorhodopsin which is being used in computer memory and processing units. Mostly halophilic bacteria and archaea have ability to degrade certain toxic compounds and have role in bioremediation of heavy metals (Horikoshi). Halophiles can also be used in biofuel production to overcome the expected shortage of petroleum and concerns regarding global warming (Ghasemi et al., 2011).

Microorganisms in the rhizospheric soil perform important functions like nutrient cycling and organic matter transformations. Metaproteomics is the study of

total community protein profile from an environmental sample. Metaproteomics 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 community from different environmental samples including ocean water, activated sludge, acid mine drainage biofilms, plant or animal tissues (Wilmes et al., 2015). Different microbial proteins and enzymes involved in metabolic pathways can be successfully characterized using MFM metaproteomics dataset. On the basis of bacterial and fungal proteins and peptides, microbial ecology can be studied. Metaproteomic approaches can also be used for restoration of contaminated and degraded soil by identification of different microbial proteins and enzymes with potential biotechnological applications (Bastida et al., 2009).

CONCLUSION

The proposed research though basic in nature, will generate enough information regarding novel biomolecules and salt tolerant genes. In addition it will elucidate the functions of organisms in soil communities. Halotolerant and halophilic bacteria have several hydrolase enzymes including lipases, amylases, proteases and cellulases. These enzymes have been used for biotechnological applications. Halophilic and halotolerant bacteria can also be used to degrade different organic compounds in bioremediation process.

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