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Advanced proteomic approaches for characterizing halophilic bacteria: Insights into protein stability and industrial applications

Pavithran Kumar [a], Pasiyappazham Ramasamy [b,*], Manjunathan Jagadeesan [a,**]

[a] Department of Biotechnology, School of Life Sciences, Vels Institute of Science Technology and Advanced Studies (VISTAS), Pallavaram, Chennai, Tamil Nadu, 600 117, India

[b] Marine Biopolymer Research Lab, Centre for Marine and Aquatic Research (CMAR), Saveetha Dental College & Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, 600077, India

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ABSTRACT

Halophilic bacteria thrive in environments with high concentrations of sodium chloride, such as salt mines, solar salterns, and hypersaline lakes. They survive extreme salinity by balancing osmotic pressure, preventing cellular damage. Extreme halophiles require up to 30 % salt for growth and structural integrity, making their proteins uniquely stable and functional in high-salt conditions. Understanding the molecular mechanisms behind halophilic protein stability is crucial for biotechnology. Investigating halotolerant proteins can lead to novel industrial applications, including enzymes for food processing, bioremediation, and pharmaceuticals. Proteomics, the large-scale study of proteins, helps characterize the proteomes of halophilic bacteria. Recent advancements in proteomic techniques allow deeper insights into protein structure, function, and adaptation in hypersaline environments. Modern methods, such as mass spectrometry and two-dimensional gel electrophoresis, facilitate the identification and analysis of halophilic proteins. This review explores advanced proteomic tools for studying halophilic bacteria, shedding light on protein stability, molecular mechanisms, and biotechnological potential. Understanding these proteins paves the way for innovations in enzyme engineering, industrial biotechnology, and synthetic biology. By leveraging cutting-edge proteomics, researchers can unlock new applications for halophilic proteins across industries, enhancing sustainability and efficiency in extreme environments.

1. Introduction

Proteins are complex molecules and play a vital role in metabolic activities in living organisms. Halophilic bacteria are an extremophilic salt-loving bacteria, they are differentiated based on their salt requirements. Halophilic bacteria are plentiful in environments such as salt pans, marine environment, and salt lakes, etc. [1]. Most of these species attribute the cytoplasmic membrane will adapt to the changes of any salt concentration in the environment. The halo adaptation of moderate halophilic bacteria will help to understand at the molecular level and prove useful in biotechnological field [2]. Comparatively very little research has been carried out in halophilic bacteria and halotolerant bacteria than haloarchaea. Haloarchaea has a unique property in cytoplasmic saline adaptation [3]. Halophilic bacteria produce hydrolysis enzymes which are tolerant in extreme salt condition. The stability of enzyme from halophilic bacteria will bind water molecules and maintain solubility and stability in high salt conditions [4].

Proteomics is an essential tool for studying bacterial mechanisms, which aims to characterize protein expression and study various stress levels in halophiles. Halophilic bacteria are negatively charged with a hydrated carboxyl group, which maintains the stable activity and adaptation of extreme conditions such as high salt concentration, temperature, and pH, etc., [5]. The expression, structure, and functions of proteins are characterized using various proteomic analytical tools such as conventional techniques involved in protein purification such as affinity chromatography, Ion exchange chromatography, and size exclusion chromatography. ELISA and western blotting are used for specific selective protein analysis. The advanced techniques of proteomics such as edman sequencing which control the amino acid sequence of specific proteins can be used [6]. For rapid protein analysis in high throughput levels, protein microarray can be used. The protein separation is done by 2-dimensional gel electrophoresis and SDS-PAGE. The 3-dimensional

* Corresponding author.
** Corresponding author.
E-mail addresses: ramkanth281@gmail.com (P. Ramasamy), jmanjunathan@gmail.com (M. Jagadeesan).

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protein structure is analyzed through two techniques such as NMR spectroscopy and X-ray crystallography. Recently, SILAC, a label-free quantification, iTRAQq quantification, and isotope-coded affinity tag labeling is used for protein quantitation. The proteomic alignment and 3D structure prediction are done by bioinformatics database analysis which provides complete structural and function of the protein [7]. Shotgun proteomics analysis is an easy and efficient method for bacterial proteomics. The protein mixture will be digested using trypsin and resulting in peptide fragments which are separated using LC-MS. The LC-MS used to analyze peptide fragment identification which is statistically analyzed by the software [8].

In the current study, the proteins which were isolated from halophilic bacteria by means of using shotgun proteomics by LC-MS was discussed. Protein expressions will analyze through mass spectrometry and bioinformatics analysis to study the function of salt tolerance and purified novel proteins will be helpful in various therapeutic applications such as anticancer, anti-coagulant, and for antibacterial purposes.

2. Halophiles

Halophilic bacteria are extremophiles that are capable of survival in the salt environment. More than 150 species are reported in halophilic bacteria. Halophilic bacteria are differentiated based on the salt concentration, they require fewer nutritional requirements and high salt requirements to grow. The major life domain of halophilic bacteria characterized as archaea, bacteria, and eukarya. Habitats of halophilic bacteria are mainly salt lakes, marine environments, salt pans, alkaline soil, soda lakes, and many other saline environments [9]. Halophile categorization was initially given and then revised. Larsen conducted the first classification study in 1962. He identified three groups of halophilic microorganisms based on the range of salt concentrations required for their best growth: weakly halophilic bacteria (between 2 and 5 % NaCl), moderate halophilic bacteria (between 5 and 20 % NaCl), and extreme halophilic bacteria (up to 20–30 % NaCl) [10].

2.1. Moderate halophiles

Microorganisms that live in conditions with a moderate salinity are known as moderate halophilic bacteria. Environments with salt concentrations between 3 % and 15 % NaCl (0.5–2.5 M) are suitable for the survival of these microorganisms. They have developed the ability to live. Moderate halophiles can endure in environments with salinities that are higher than those found on Earth, but lower than those that extreme halophiles prefer. Cellular Adaptations of moderate halophilic bacteria which balance the osmotic pressure and avoid dehydration, these bacteria accumulate suitable solutes like potassium ions (K^+), glycerol, and sucrose. This allows them to maintain cellular integrity in saline environments [11].

Moderate halophile enzymes frequently need a lot of salt to function at their optimum. For example, salts, particularly potassium, activate several of their enzymes. Morphology of moderate halophiles, in contrast to extreme halophiles, they typically have a fairly normal cellular structure, though they may exhibit changes in the structure of their cell membranes to avoid excessive salt buildup or dehydration. Moderate halophiles typically inhabit environments with salinities between 3 and 15 %, such as salt marshes, salt ponds, and hypersaline lakes and also present in saline sewage treatment plants, certain mild halophilic species can be employed in bioremediation procedures [12].

2.2. Extreme halophiles

Extremophilic bacteria, also known as extreme halophiles, are organisms that need extremely high salt concentrations to grow as efficiently as they can. These bacteria are adapted to conditions where the salinity exceeds 15 % NaCl (approximately 2.5 M), and they may even live in environments with salt concentrations beyond 30 %, such as salt lakes or brine pools. These high salt levels are frequently necessary for their metabolic and structural integrity. Extreme halophilic bacteria have cellular adaptations that enable them to survive in extremely salinized conditions without losing cellular structure. These adaptations are found in their cell walls, which include special chemicals. By keeping water in the cell, these modifications help avoid dehydration. Extreme halophiles build up large amounts of potassium chloride to assist with osmotic pressure; in rare cases, their cells contain 4–5 M Kcl. [13]. Extreme halophiles have highly salt-dependent enzymes. These proteins frequently need a certain ionic composition to remain active because they are designed to work in high ionic conditions. The metabolism of certain severe halophiles can make use of a variety of organic and inorganic substances (such as sulphur for respiration or light for photosynthesis). Usually, their metabolic processes have been modified to function well in salinized settings [14].

Extreme halophiles can be found in locations such as Lake Urmia, the Dead Sea, and the Great Salt Lake in Utah. Most species cannot survive in the hypersaline waters of these lakes. Extreme halophiles thrive at the extremely high salinity that is frequently maintained in commercial salt production facilities. When the salinity is really high, extreme halophiles can occasionally be found in salt crystals. The example of extreme halophilic bacteria such as species of *Halobacterium* Among the most researched is *Halobacterium salinarum*, which is employed in a number of research projects, such as investigations into the diversity of microorganisms in harsh condition. Some strains of the *Haloferax species*, which are prevalent in hypersaline conditions, are used in biotechnological processes such the synthesis of enzymes. Because they can withstand both salinity and alkalinity, *Natronomonas species* bacteria are frequently discovered in soda lakes with a high pH and high salt content [15].

Halophilic bacteria have vast Biotechnological applications such as Pharmaceutical, environmental, and other industrial approaches. In order to survive in extreme conditions, halophilic microbes have evolved special defense mechanisms and metabolic activities. The capacity to create unique exopolysaccharides is one of these tactics. Exopolysaccharides from halophilic bacteria generated enthusiasm because of their particular rheological, physiological, and structural characteristics. Because of their varied composition, size, and structure, Exopolysaccharides are important. Therefore, it is intriguing to investigate and discover the great variety of exopolysaccharides characteristics and uses, as well as their possible uses in paint, petroleum, food, medicine, cosmetics, textiles, pharmaceuticals, agriculture, and wastewater treatment [16].

3. Halophilic enzymes

Halophilic enzymes play multiple roles in biotechnology field. Most of the halophilic bacteria are hydrolysis enzymes which are tolerance of extreme conditions such as salt concentration, temperature, and other extreme conditions. Protease enzymes produced by various halophilic bacteria such as *Halobacillius karajensis* [17], *Bacillus aquimaris* [18] with high stability in saturated salt concentration which plays an economically important role of extremozymes in pharmaceutical and industrial processes [19]. α-amylase is an endoenzyme reported from several halophilic bacteria including *Aspergillus gracilis* [20], Marinobacter sp. [21] & *Halomonas meridiana* [22]. β-amylase is an exoenzyme reported from *Salimicrobium halophilum* strain LY20 [23] & *Halobacillius* sp. LY9 [24], which are an advantage to achieve optimal activities and solvent stability at extreme conditions [25]. Lipase is an important hydrolytic enzyme which plays a significant role in the detergent formulation, pharmaceutical, and industrial processes because of solvent stability at a high salt concentration which is reported from *Pseudomonas stutzeri* [26], *Piscibacillus ish* [27] & *Bacillus* sp. *ORS4* [28]. Cellulose plays multiple roles in industrial biotechnology such as pulp and paper, fabric material, and food processing [29]. Most of the Cellulose production from halophilic isolate which is good in biofuel production such as *Haloarcula* sp. Strain LLSG7 & *Bacillus methylotrophicus RYC01101* [30].

Table 1
List of Halophilic bacteria with their Enzyme production activity.

Isolate	Salt tolerance %	Enzyme	Production activity U/ml	Reference
Haloferase sp. HA10	15	Amylase	4.2	[31]
Halobacillus sp. HA4	15		5.6	
Haloalkaliphilic bacterium EMB3	6	Protease	21	[32]
Bacillus luteus H11	6	Protease	115.2	[33]
Halobacillus sp. SP4	12	Lipase	3.7	[34]
Bacillus aryabhattai	8.6	L-glutaminase	46.4	[35]
Bacillus sp. BCCSo34	7	L-asparaginase	1.64	[36]
Gracilibacillus sp. SK1	10	Cellulase	107.8	[37]
Halobacillus sp. K51	15	Pectinase	55.8	[38]

Halophilic bacteria capable of producing various halophilic enzymes are shown in Table 1.

4. Proteomic studies on halophilic bacteria

Proteomic studies are an essential tool for studying the mechanism of bacteria. Proteomic research on halophilic bacteria is very less. The proteomics of halophilic bacteria aims to characterize (1) protein expression map, (2) to study and understand the various stress conditions of halophiles. All halophilic proteins are highly negative charged with hydrated carboxyl group which maintains the protein solubility at high salt concentration. The proteins of halophilic bacteria are active and stable adaptation in high salt concentration conditions [39]. Investigating the molecular levels of proteins, mRNA, and important metabolites is crucial. Researchers found that a hybrid approach, combining the "compatible solute" and "salt-in" processes, was used for osmotic adjustment during the long-term salinity adaption of halophiles based on proteome profiling of halophilic bacteria conditions. This conclusion is supported by the intracellular concentration of K+ and compatible solutes, as well as the mRNA level of important proteins. To adapt to high salinity, halophilic bacteria specifically use the glutamate and proline production pathways, the glycine betaine ABC transporters (Opu and ProU families), and the Na+/solute symporters. In extreme halophilic bacteria, the internal concentration of suitable solutes, such as glutamate, proline, and glycine betaine, rises as salt levels do. The maintenance of intracellular K+ concentration is further made easier by the overexpression of Na+/K+/H+ transporters, which guarantees cellular ion homeostasis throughout a range of salinities. Moreover, *N. thermophilus* reacts to elevated Na + concentrations by exhibiting cytoplasmic acidification. As salinity rises, the upregulated proteins' median isoelectric points fall. The ability of *N. thermophilus* to adapt to high salt stress is facilitated by bacterial chemotaxis, membrane transport, amino acid metabolism, and energy and carbohydrate metabolism [40].

Proteomics method is classified into two concepts, (1) Top-down proteomics, which are intact proteins with high-throughput analysis (2) Bottom-up proteomics are identified and characterize the peptide fragments from digested protein [41]. In the year 2002, Top-down proteomics study rooted to target research, cooperation, and facilitate the intact protein analysis. Top-down proteomics studies the different biological functions of protein [42]. Top-down proteomics will identify and describe the protein networks using direct fragmentation. This mechanism protects the post-translational modification of proteins. In clinical biochemistry, Top-down proteomic techniques provide the modified protein probe as a biomarker, which helps to diagnosis the various diseases [43]. There are many applications in proteomic research using Top-down proteomic analysis. The protein separation in Top-down proteomics aims for standard separation of proteomics. In this intact protein separation, there are around 30 KDa become a regular process in proteomic analysis in mass spectrometry (Fig. 2). This will useful for the sample preparation, instrument analysis, and improve the additional methodological development [44]. In bottom-up proteomics, the protein identified from the tissue sample by increasing the peptide using polarity-based fractionation [45]. In clinical research, bottom-up proteomics study has the potential to provide protein network analysis which specially focuses on cardiovascular research [46]. In a bottom-up proteomic study, developed new UHPLC system which has more advanced levels for the protein analysis such as a high number of peptide identification, High sample loading capacity and quantified proteins [47].

4.1. *Halophilic proteins structural and physicochemical modifications for survival in high-salt conditions*

In contrast to their non-halophilic bacterial homologs, halophilic bacterial proteins exhibit strong compositional and surface charge biases and are stable in their functional molar concentrations of NaCl and KCl. The highly negative surface potential, long-range electrostatic repulsion, low aggregation tendency, and strong interaction with hydrated ions are characteristics of halophilic bacterial proteins, which are substantially enriched in low isoelectric points and acidic residues. The

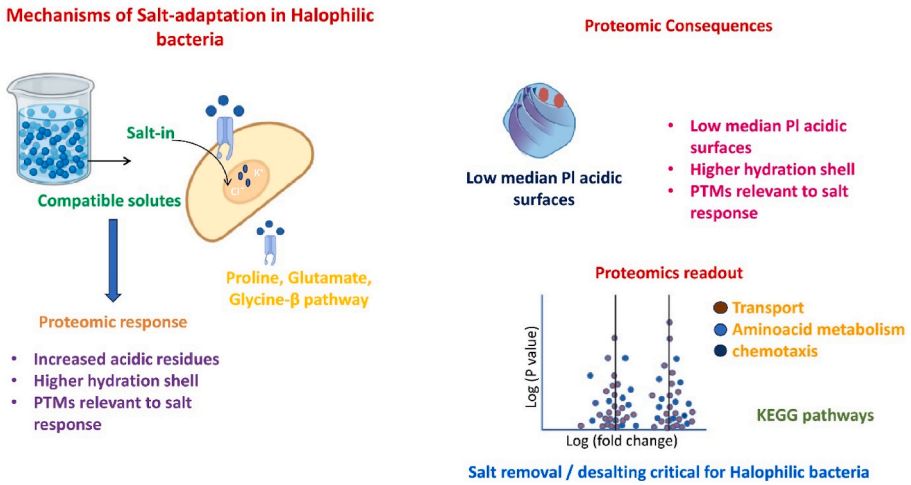

Fig. 1. Mechanism of salt adaptation of halophilic bacteria.

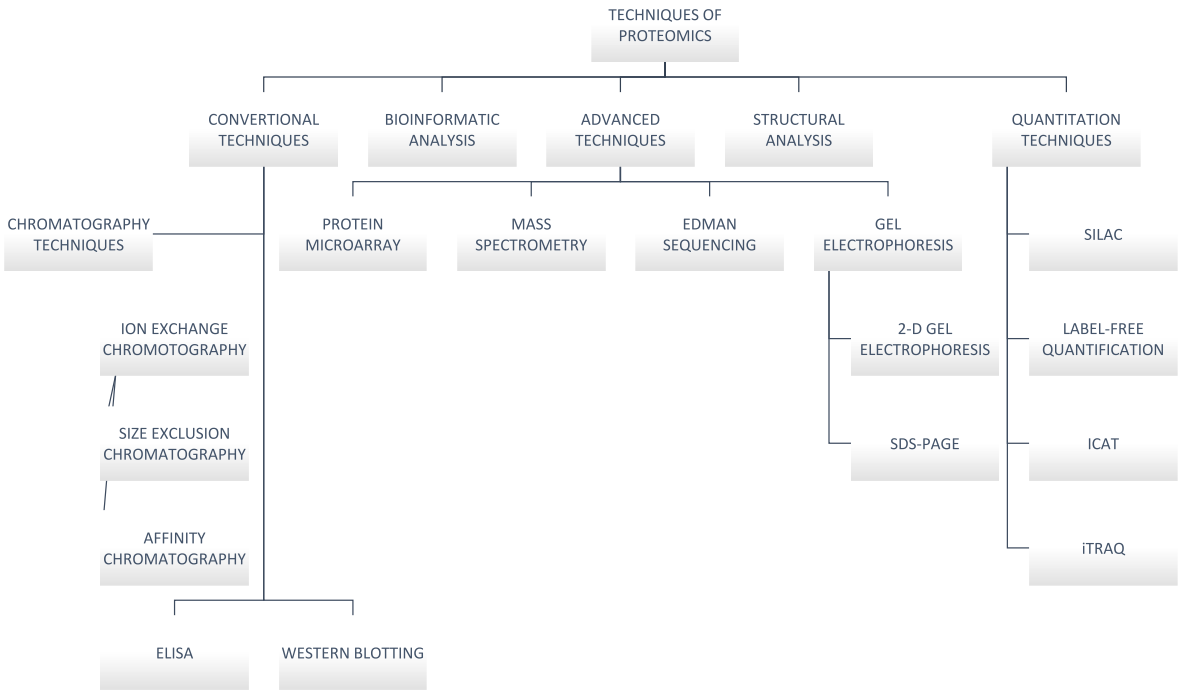

Fig. 2. an overview of proteomic techniques.

initial stages of severe halophilic bacteria are these acidic halophiles [48]. The solvent structure will vary in a high-salt environment due to decreased hydrophobic interactions with water molecules, which will lower hydrophobic density, reduce interactions between hydrophobic residues and polar residues, and weaken ion-mediated stabilization. Halophiles and acidic surface K+ ions interact with proteins to form dynamic ions that preserve folded states, weakening the hydrophobic core and preserving the saltiness that denatures the protein [49]. The proteomic implications will increase the frequency of acidic residues in halophilic bacteria, enhance the expression of proteins involved in the biosynthesis of osmolytes and ion transporters, and shift the fold distribution to reflect adaptation to solvent energetics. These characteristics will be compared to the mechanism of salt tolerance in halophilic bacteria and quantitative proteomics [50] (Fig. 1).

4.2. Ion exchange chromatography

Ion exchange chromatography is an analytical technique for protein purification based on their charge group (anion & cation) on its surface [51]. The Halocins producing protein from *Halobacterium salinarum*, the protein has antimicrobial property against multiple bacteria [52]. The halophilic alpha amylases enzyme from different halophilic bacteria was purified using anionic exchange chromatography, these proteins are performed in food processing such as hydrolysis products [53]. The xylanase from halophilic bacterium -OKH purified in ion-exchange chromatography and reported has it has potential biotechnological applications [54].

4.3. Affinity chromatography

The affinity chromatography separates the biochemical mixture based on their specific interaction between enzyme & substrate and receptor & ligand. In protein, purification explores protein-protein interaction and post-translational modifications [55]. The poly-extremotolerent laccase was purified from halophilic bacterium *Aquisalibacillus elongatus* by affinity chromatography. The enzyme proved to be valuable for industrial applications since it effectively delignified sugar beet pulp in an ionic liquid [56]. Various ligands, antibodies, and plasma proteins are purified by affinity chromatography which is applicable for various therapeutic uses and industrial processes [57,58].

4.4. Size exclusion chromatography

Size exclusion chromatography is a powerful analytical technique for protein purification which separates the protein based on their size and molecular weight. Mostly significantly size exclusion chromatography purifies the non-covalent multimeric proteins. The *Spiribacter salinus* produce the lipopolysaccharides like soluble factor was purified through size exclusion chromatography exhibited more fractions. The several antimicrobial peptides from halophilic bacterium were purified through size exclusion chromatography which inhibits the pathogens in skin infection [59,60].

4.5. ELISA

Enzyme-linked immunosorbent assay is a sensitive immunoassay technique and commonly used in detection of enzymes and also diagnostic use [61]. Detection of enzymatic assay of salt-induced activation of methionine sulfoxide reductase from the halophilic bacteria *Halobacterium hubeiense* using ELISA. Which clarify the molecular Basis of halophilic enzyme activity in reaction to salts [62]. A potent method for identifying halophilic bacteria is ELISA, which looks for particular antibodies or antigens linked to these microbes. It is useful for quick detection in environmental samples because to its excellent sensitivity and specificity, particularly in saline or hypersaline environments. ELISA is frequently used in biotechnology applications requiring saline conditions, food safety testing, and water quality monitoring. Its non-invasiveness makes it possible to monitor halophilic bacterial populations in a variety of habitats effectively and economically [63].

4.6. Western blotting

Western blotting is a proteomic technique that detects the protein, where the protein separated by using electrophoresis through

nitrocellulose membrane [64]. Hiroko et al. created a HrdC expression vector, pHS-hrdC, using a *Halomonas-E. coli* shuttle vector, pHS15, and conjugated it into strain 160 cells to overproduce the HrdC protein. Western blotting with an anti-GST-HrdC antiserum to analyze the protein profile of the outer membrane of 160(pHS-hrdC) showed that the amount of HrdC protein in 160(pHS-hrdC) was almost four times greater than that in 160. The antibiotic susceptibility profile of the overproducer cells was identical to that of wild-type cells, according to our analysis of the antibiotic susceptibility of the cells that overproduced HrdC [65]. By introducing the bla gene into the halophile-E. coli shuttle vector pHS15, a HaBlap expression vector, pHS15-bla, was created. It was then conjugated with an E. coli mobilizer strain that carried pHS15-bla to introduce it into strain 560. It was discovered that the β-lactamase activity of the transformant 560(pHS15-bla) cells was approximately five times that of the 560(pHS15) cells. Using the anti-GST-HaBlap antiserum used for this investigation, western blotting of HaBlap After the gene bla was cloned, it was determined that the enzyme's main structure was particularly abundant in acidic amino acid residues, which is a feature of halophilic proteins. The enzyme is halophilic, however after heat denaturation, it refolds in low-salinity environments [66].

4.7. Edman sequencing

Edman sequencing is a chemical reaction method in an amino acid peptide or protein sequencing. Edman sequence widely plays an important role in therapeutic proteins [67]. The cultures of *Halobacterium salinarum* ETD5, *Halobacterium salinarum* ETD8, or *Halorubrum chaoviator* ETD3 were used to make intracellular protein extracts. This protein was estimated in SDS PAGE electrophoresis and the bands were incubated with agitation in 100 mM Tris-HCl, 3.5 mM SDS, 100 mM NaCl, and pH 8 transfer buffer for a whole night at 37 °C in preparation for edman sequencing. Before being subjected to protein sequencing, a polyvinylidene fluoride membrane was activated by methanol for 60 s, washed, and incubated with the bands for 24 h at 37 °C to facilitate peptide transfer [68]. Degradation of N-terminal tripeptide using edman activity by glycoprotein process cleavage of amino acids involved in the several applications of the halophilic bacteria [69].

4.8. Analytical protein microarray

Protein microarray or protein chip is an important proteomic technique which detects the proteins by direct protein labeling which measure the protein level of expression and protein binding affinities [70]. Halophilic bacterial proteins that react to salinity changes can be profiled using analytical protein microarrays. The identification of salt-responsive biomarkers is made possible by these microarrays, which offer a thorough picture of the proteome at various salt concentrations. Gaining knowledge about how salt stress affects halophilic proteins can help us understand how microbes adapt. Applications in synthetic biology, environmental microbiology, and even the creation of organisms for bioremediation or crops resistant to salt may depend on this information [71].

4.9. Two-dimensional gel electrophoresis

The 2-dimensional gel electrophoresis is an important technique for protein separation based on their size and molecular weight which characterize the post-translational modification and metabolic pathways [72]. Two-dimensional gel electrophoresis (2-DE) has inherent issues with sample preparation and protein separation from *Halobacterium salinarum*. Because of the high salt content, proteins derived from cells cultured with 25 % NaCl are particularly challenging to resolve using 2-DE. A 3 kDa molecular weight cut-off column was employed to exclude salts. The majority of the soluble proteins were concentrated in the acidic range when they were separated using 2-DE. Ultrazoom immobilized pH gradient strips were utilized to separate proteins in the pH 3–6 range. Furthermore, the proteome analysis of acidic proteins was better accomplished by sample separation utilizing an IPGphor/Multiphor combination system than by employing IPGphor for the isoelectric focusing stage [73]. The *Halobacillus dabanensis* metabolic system characterizes the bacterial protein regulation that was identified by 2-D PAGE [74].

4.10. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

SDS-PAGE is a proteomic tool for protein separation based on their molecular weight. Denaturation of protein using sodium dodecyl sulfate, separate based on their molecular weight [75]. The isolated AMP from halophilic Bacillus firmus had a molecular weight of 36 kDa, according to SDS-PAGE examination, and it possesses antibacterial properties [76]. Using SDS-PAGE, the expression of the ectoine proteins from four distinct halophilic bacteria was found, and the cultures' antibiotic resistance was profiled using the disc diffusion method [77]. The bacteriorhodopsin proteins from *Halobacterium sodomense* and *Halobacterium salinarum* pigments was estimated in SDS_PAGE [78]. The protein profiling of 24 different halophilic bacterial proteins were characterized through SDS-PAGE, which performs in large taxonomic research [79].

4.11. X-ray crystallography

X-ray crystallography is an important proteomic technique that determines the protein 3-D structure which provides more information in enzyme mechanism and protein-ligand interaction [80]. The 3D structures of halophilic bacterial proteins, which frequently have distinctive structural characteristics that allow them to operate in high-salinity conditions, may be well understood at the atomic level thanks to XRD crystallography. For instance, in order to preserve stability and avoid denaturation under high ionic strength, these proteins usually contain a larger percentage of acidic residues, more charged surface areas, and occasionally a more rigid protein structure. Because halophilic proteins are extremely flexible and occasionally disordered, they frequently have trouble crystallizing, especially when high salt concentrations are present. These proteins require a special chemical environment (such as certain salts or buffer conditions), which might make crystallization difficult. XRD is still a crucial method for overcoming these obstacles, however it frequently necessitates the creation of new procedures for halophilic proteins as well as the optimization of crystallization conditions (temperature, pH, and salt concentration). The potential of XRD for halophilic protein research is being further expanded by developments in cryo-XRD and other methods, like in situ crystallography, to investigate these proteins in their natural high-salinity settings [81,82].

4.12. NMR spectroscopy

One analytical method for studying structure, kinetics, and protein analysis is NMR spectroscopy. NMR will examine molecular-level haloadaptation in halophilic proteins, which confers stability and solubility at high salt concentrations [83]. To examine the native structures of halophilic bacterial proteins under high-salt conditions and to monitor ionic conditions during folding, NMR will be used. The halophilic protein with less compact hydrophobic cores will be studied by NMR [84]. High salt concentrations will disrupt the traditional water structure and, as a result, decrease hydrophobic interactions. To characterize halophilic proteins that interact with hydrated K+ ions, NMR will also reveal extensive acidic surface patches. The structural and ionic environment alterations are the outcomes of the salt titration NMR [85]. The mechanism of halophilic bacteria depends on these acidic protein surfaces, ion-binding sites, and salinity. identification of residues' salt tolerance, ion-binding interaction mapping, and function adaptation in high salinity [86,87].

4.13. Mass spectrometry

Mass spectrometry is an important proteomic analytic technique that measures the mass to the ratio (m/z) and protein molecular weight determination [87]. In clinical research, the identification of bacteria such as growth and anaerobic bacteria was identified through the MALDI-TOF technique and also identify the pathogenic bacteria which help to study the disease control [88]. When studying halophilic bacterial proteins, mass spectrometry (MS) is a useful technique that allows for accurate protein species identification and quantification in high-salt environments. It aids in the characterization of protein-protein interactions and post-translational changes that are essential for salt tolerance. Proteome profiling and the identification of halophilic bacterial biomarkers are more applications for MS. Applications of this approach can be found in biotechnology, where salt-stable proteins are needed for processes like enzyme discovery and bioremediation [88]. The tissue proteins can be analyzed by MALDI Mass spectrometry which detects drug administration and metabolites and also study the toxicological and therapeutic process [89].

4.14. Bioinformatic analysis

Bioinformatics plays a major role in proteomics for data analysis. This arising field introduces a novel algorithm to various proteomic data [90]. Endolysis is a therapeutic enzyme which control the spreading of multi-drug-resistant bacteria. The therapeutic bacteria have been modified using the DNA shuffling method [91]. The protein domain and amino acid sequence arrangement are studied through multiple sequence alignment (MSA) [92]. The identification of halophilic proteins has gained significant attention in research due to the vast array of possible uses for these proteins. Using whole-genome sequencing and wet laboratory validation techniques to screen for halophilic proteins, numerous research has gathered samples of halophilic bacteria and archaea from salt lakes and oceans across the world. Indeed, the finding of numerous protein sequences from a wide range of organisms has been made easier by advancements in DNA sequencing technologies, especially the application of genomics and metagenomics methods. Researchers discovered that while the majority of the proteins in the halophilic protein database HProtDB6 originate from halophilic bacteria, proteins generated from these bacteria might not be halophilic proteins. The intracellular salt concentrations of certain halophilic bacteria, such halobacterium, can be efficiently controlled to be lower than those of the extracellular environment [93,94]. To balance the osmotic pressure within and outside the cell, they can also produce and collect substances called suitable solutes, such as proline, lysine, and ectoine. Consequently, some non-halophilic proteins may be present in these bacteria. This problem is anticipated to be resolved by computational approaches for halophilic protein screening, which have garnered more and more interest from researchers. However, performance generalization and a significant lack of data plagued earlier approaches. Additionally, the public's direct access is hampered by the absence of user-friendly software or websites. To help harness the potential of halophilic proteins, a strong, more accurate tool that is easily accessible to the general public is therefore required [95]. Shantong et al. developed a sizable and thorough dataset of halophilic proteins and presented an innovative approach to data collection. built on this, presented a machine learning (ML) model for identifying halophilic proteins called Halophilic Protein Classifier (HPClas), which is built on catBoost. High-quality data from the NCBI protein database and UniProtKB were used to train HPClas. This model can determine whether a protein is halophilic based on inputs such as amino acid sequences. On the test set of an earlier work, we evaluated HPClas's performance against that of state-of-the-art (SOTA) models. According to experimental results, HPClas performed better than the current methods for predicting halophilic proteins. Additionally, research carried out an interpretation analysis of the model, which strengthened the validity of our forecasts [96]. Genomics and metabolomics include proteomic technologies to develop powerful tools for algorithm development. Protein identification and characterization help to find the new algorithm for data analysis and possible to study the protein expressions at a high level.

5. Proteomic quantitative techniques

The quantitative techniques of Halophilic bacterial Proteomics are majorly differentiated in following types of analysis.

5.1. SILAC

Stable isotope labeling by amino acid is the most important quantitative proteomic method. SILAC advances towards the mechanism of endogenous translation of living organisms which labels the chemical analogs of DNA building blocks [97]. The classical SILAC, the specific-site dynamic of histone acetylation and methylation has been adapted to study the chromatin modification. The chromatin modification will provide an effective biomarker labeling through LC-MS [98]. The advanced SILAC quantitative characterize steady-state kinetics of methylation and acetylation approach gene regulatory studies in different metabolic conditions [99]. More than 1500 proteins were identified and quantified from *Bacillus subtilis*, most of the genes are expressed in log phase. In this, SILAC quantitative proteomic analyzed 10 to 30 phosphorylation sites under phosphate starvation and succinate growth [100]. Oxidative stress responses are linked to lysine acetylation, according to research on the halophilic archaeon *Haloferax volcanii*. The potent oxidant hypochlorite: (i) promotes a rise in the abundance ratios of the lysine acetyltransferase HvPat2 to HvPat1 and (ii) favors lysine deacetylase sir2 mutants. Here, their describe how the lysine acetylome of *H. volcanii* cultured in glycerol changes in profile in response to hypochlorite, demonstrating its dynamic occupancy. Quantitative multiplex proteomics of the Δsir2 mutant strains and the SILAC-compatible parent strains reveals these results [101]. SILAC analyzed more than 600 intracellular stability proteins in human adenocarcinoma cells which applicable in tissue engineering to regenerate the cells and tissues [102].

5.2. Label-free quantification

Label-free proteomic quantitative analysis is the high throughput semi-quantitative analysis. Most label-free quantitative analyze the clinical proteomics in large size quantity samples which quantify and output the high dynamic range yield [103]. There is a smaller number of samples were analyzed in computational base label-free quantification. In the future, LC-MS coupled label-free quantification analysis provides an efficient yield [104]. Label-free quantification relies on spectral counting quantification, peptide fragment will higher in sample analysis under chromatogram of a mass spectral [105]. When it's essential to research proteins without adding labels that might alter their natural state or activity, label-free quantitative approaches are frequently employed for halophilic bacterial protein applications. These methods shed light on relationships, post-translational changes, and protein abundance in complex materials. Numerous pieces of evidence demonstrate that the development of N-glycan on related proteins is necessary for organism to respond adaptively to salt stress. Nevertheless, little is now understood about the salt-responsive glycoproteins involved in this process. In this investigation, we found that wild-type (WT) Arabidopsis and two mutants, mns1 mns2 and cgl1, that are impaired in N-glycan maturation have salt-responsive glycoproteins. Label-free liquid chromatography coupled mass spectrometry (LC-MS/MS) quantitative analysis revealed 97 proteins with abundance variations of >1.5- or <0.67-fold against salt stress [106].

5.3. ICAT labeling

Isotopic-Coded Affinity Tag is an isotopic labeling method for

analyzing protein sequence identification and quantification using chemical labeling reagents which output accurate results [107]. The preferred technique for precisely identifying shifts in protein abundance in extremely complex mixtures is quantitative proteomics based on isotopic labeling. Three distinct cellular states of the halophilic archaeon *Halobacterium salinarum* were compared pairwise using isotope-coded protein labeling (ICPL), a straightforward, dependable, and quick technique based on the nicotinoylation of proteins at lysine residues and free N-termini [108]. The biotic and abiotic stress levels under reactive oxygen and nitrogen species of many organisms were carried out in ICAT proteomics. The combination of mass spectrometry and ICAT provides an efficient result. The tumor-specific protein from breast tumor patients, the vitamin-D binding protein were expressed which approach biomarkers for disease diagnosis [109].

5.4. iTRAQ

Isobaric tag for relative and absolute quantification is based on tandem mass spectrometry for proteomic quantification. iTRAQ techniques based on protein labeling with isobaric tags in N-terminus and amine group through mass spectrometry [82]. *Lactobacillus plantarum* is a salt-tolerant probiotic strain. After salt stress, we used isobaric tags for relative and absolute quantitation (iTRAQ)-based proteomics and bioinformatics analysis to examine the underlying molecular pathways of L. plantarum. Salt stress, both mild and high, was applied to L. plantarum. Proteins with differential expression in each group were quantified using iTRAQ's quantitative proteomic method and LC-MS/MS. Moreover, KEGG carried out bioinformatics study, and Mascot and GO identified DEPs. Mapped and categorized into nine functional groups were thirty DEPs ($P < 0.05$) between low salt stress and control condition (0 % (w/v) NaCl); 122 DEPs between high salt stress and control condition were mapped and categorized into fifteen functional groups. the transporter of the ATP-binding cassette (ABC) and metabolism [110]. The adaptive reactions of *H. salinarum* NRC-1 to changes in salinity, the proteome was characterized using iTRAQ. It was demonstrated that 399 proteins with differential expression were linked to variations in the external sodium chloride concentration, with metabolism-related proteins exhibiting the largest response [111].

5.5. Alpha-fold predicted 3D proteins

Evolution depends on proteins, and knowing their structure can help us understand how they work mechanistically. There are billions of known protein sequences, yet only about 100,000 distinct protein structures have been identified through massive experimental effort. Determining a single protein structure takes months to years of meticulous work, which limits structural coverage. For large-scale structural bioinformatics to be possible, this gap must be filled with precise computational methods. For almost 50 years, the structure prediction component of the "protein folding problem" has been a significant open research problem. It involves predicting the three-dimensional structure that a protein will adopt based only on its amino acid sequence. Even with recent advancements, current techniques are still far from atomic accuracy, particularly in the absence of a homologous structure. The first computational technique that can consistently predict protein structures with atomic accuracy—even when no comparable structure is known—is shown here. In the difficult 14th Critical Assessment of protein Structure Prediction (CASP14), we validated a completely redesigned version of our neural network-based model, AlphaFold, which significantly outperformed other approaches and showed accuracy competitive with experimental structures in most cases [112,113]. The most recent iteration of alphaFold is based on a revolutionary machine learning technique that uses multi-sequence alignments to integrate biological and physical facts about protein structure into the deep learning algorithm's architecture. With an emphasis on the Cas9 protein of the halophilic bacterium *Salicibibacter cibi*, this work investigates the structural modifications of the CRISPR–Cas9 system in halophilic bacteria. Several technologies, including the predictor of natural disordered areas online service for disordered areas, the InterPro server and WebLogo for domains, and ExPASy ProtParam for various physicochemical parameters, were used to examine protein sequences. The AlphaFold database was used to construct protein structures, and PROCHECK was used to assess the modelled structure's quality. PyMOL, APBS server, and UCSF chimera were used to visualize the electrostatic potential and amino acids on the protein surface. According to comparative study, halophilic Cas9 proteins exhibit more inherently disordered areas and a greater abundance of acidic residues, which improve stability and hydration in saline environments. The maintenance of a significantly negative surface charge by *S. cibi* Cas9 proteins, which is essential for adaptation to salt-rich environments, was verified by electrostatic potential maps. These results highlight the potential uses of Cas9 in genome editing-based biotechnological techniques in harsh environments and shed information on the molecular mechanisms behind its structural and functional changes in salty environments [114].

5.6. Shotgun proteomics

Shotgun proteomic analysis is an easy and efficient method for bacterial proteomics. A high-throughput method for analyzing complicated protein mixtures, such as those from halophilic bacteria, is shotgun proteomics. Proteins are broken down into peptides, which are subsequently subjected to mass spectrometry (MS) analysis in order to identify and measure proteins according to their peptide sequences. Because this approach can manage the varied and salt-adapted proteins seen in halophiles, it is very helpful for researching their proteome. A thorough grasp of how halophilic bacteria react to extremely high salinity levels is made possible by shotgun proteomics. The process of shotgun proteomic start with protein mixture which is digested using trypsin and resulting in peptides are separated using HPLC or LC-MS. Peptides are identified by mass spectrometry [115]. There are a variety of methods for sample preparation. Mostly sample preparation will vary, which all depends on their respective advantages and disadvantages. In this study the least amount of processing to produce the maximum amount of data. Protein detection and quantification also have a variety of ways to process the proteomic study. For protein lysis most of the studies shown that protein extract was used to lyse with diothiothreitol and iodoacetamide, which are disulfide reducing agents will prevent the intramolecular and intermolecular disulfide bonds process between cysteine residues to protein [116]. In one of the study, lithium dodecyl sulfate buffer was used as a density gradient for protein lysis, and showed that this will not affect the protein and their solubility and also stable in alkaline and neutral solutions [117].

Usually, the proteomics studies require both identification and quantification of protein. The protein was used to separate and quantify using two - dimensional gel electrophoresis. In shotgun proteomic experiment the protein mixture was digested and analyzed in mass spectrometry [118]. There are two fundamental approaches for quantification of protein such as *in vivo* and *in vitro* labeling. The *in vitro* method is based on the chemical and enzymatic process to derivate the functional group [119]. The *in vivo* method is the biosynthetic process that incorporates isotope-labeled precursors [120]. SDS PAGE is widely used for protein fractionation and it was analyzed in mass spectrometry. A study showed that SDS PAGE was performed and the gel slices were dehydrated with acetonitrile and ammonium bicarbonate which enhance the peptide - peptide hydrogen bond and improve the pH stability [121]. Alkalyation plays an important role in the proteomic experiment, iodoacetamide is an alkylating reagent that cleaves the disulfide bonds of a protein without the addition of disulfide reducing agent [122,123]. Reported that iodoactamide shows a good result for protein alkylation compared with other alkalotic reagents to maximize peptides identification. Protein digestion was used to improve the

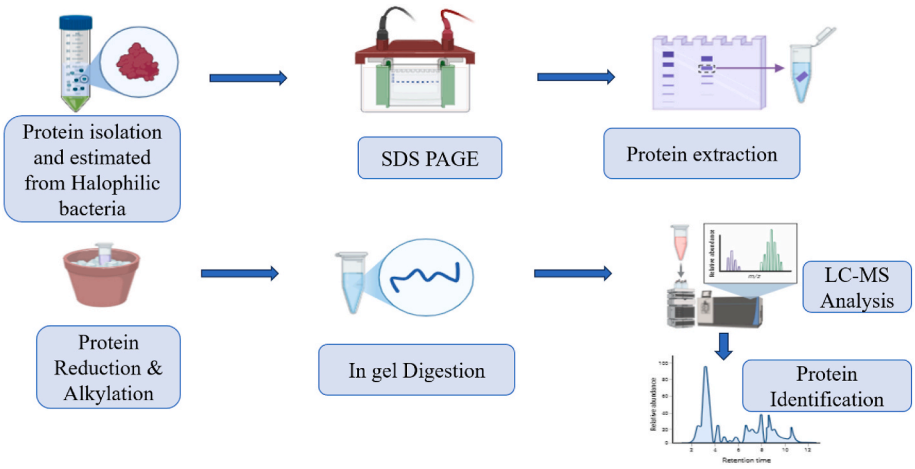

Fig. 3. Shotgun Sequencing of Halophilic bacterial protein.

proteomic sequence coverage and identification of post-translation modification [124]. Protein digestion mostly performed with trypsin, because of their high specificity. In the study by A.C. Vergunst et al., discussed the trypsin from digested protein into peptides fragments are separated based on the hydrophobicity process using reverse phase column chromatography and the molecular weight is quantified by mass spectrometry [125] (Fig. 3).

In shotgun, proteomics has multiple applications. The proteins generated from the Human dentin using shotgun proteomics, the number of proteins with their functions which are applicable in tissue engineering to regenerate the dental pulp [126]. The shotgun proteomic analysis of Halophilic bacteria, the protein with their physiological functions which perform as biomarkers, provide nutritional development and disease resistance [127]. Shotgun proteomics applicable for drug discovery & drug identification and also the identification of drug targets. The proteins serve as a biomarker of human disease which acts as a therapeutic diagnostic tool to treat various diseases [128].

5.7. *Mass spectrometry & LC-MS for peptide analysis*

Mass spectrometry is a proteomic tool for the identification and quantification of the proteome of an organism. There are different methods of mass spectrometry technologies used for the analysis of peptides. Tandem mass spectrometry is an effective analytical tool for proteomic studies. In this method, there is two trends process, the first trend is linear ion trap mass spectrometry is often used tool for peptide sequence identification. This tool has high ion capacities than the older 3-D ion traps, which are sensitive and provide quality data. The second trend is a modified instrument with improved capabilities for proteomic studies, joined with quadrupole mass filters provides capable ion trap of tandem mass spectrometry. Fourier transform ion cyclotron resonance (FTMS) is a powerful proteomic analytical tool, the ability to perform routine, and access the acquire high-quality data [129]. A potent method for describing halophilic bacterial proteins, LC-MS peptide analysis makes it possible to identify and measure complicated proteomes. Peptides are separated by liquid chromatography following protein extraction and enzymatic digestion, and their mass-to-charge ratios are subsequently examined by mass spectrometry. High sensitivity and resolution are provided by this technique, which is crucial for researching the salt-adapted proteins found in halophiles. It makes it easier to comprehend post-translational changes, protein activities, and adaptations to extremely salinized environments.

In bottom-up proteomics, protein extracted mixture was digested by a protease into peptides based on their physical or chemical properties. The peptides mixture is analyzed in mass spectrometry for protein identification. There are two fundamental approaches for the bottom-up mass spectrometry proteomics studies are (1) identification of protein from the sample, (2) quantification of levels of the identified proteins [125]. Mass spectrometry will select the peptide ions these ions are subjected to fragmentation, which resulting the fragment ion spectrum are recorded and provides the peptide signature which is identified by a sequence database search algorithm tool [126].

Liquid chromatography combined with mass spectrometry (LC-MS) is an important analytical tool for proteomics experiments, which contribute a qualitative and quantitative analysis of different proteins in the high-throughput pattern. This method used for the synthesis of quantitative information and protein quantity detection [127]. Most of the studies showed that LC-MS is mostly used peptide analytic method in shotgun proteomic studies [121]. A nano LC-MS is a proteomic analytical tool for the analysis of peptides, which characterize the entire complements of proteins in the organism. The resulting peaks are identified and quantified using software tools.

5.8. *Data processing of protein*

Generated data are collected from the mass spectrometry are analyzed to extract the information. There is much software for the identification of proteins. In shotgun proteomics, tandem mass spectra are identified from the sequence database and make amino acid sequences. Many algorithms will fulfill this process to measure and amino acid sequence determination to fits the mass spectra to sequence the algorithm to score the functions with cross-connection analysis provides final measure with the proximate fits [124]. The peak list from mass spectrometry is identified using mascot daemon software (version 2.1.10 matrix science). The identified proteins are included in the spreadsheet to spectral count each protein values. The spectral count values are compared with LC-MS/MS run which is statistically analyzed in software [115].

6. *Halobacterium* species

Halobacterium salinarum an extremely halophilic archaeon protein is identified using tandem mass spectrometry and bioinformatic analysis. Among 29 proteins of *Halobacterium salinarum*, vng 0487h gene selected for recombination. The purified recombinant vng 0487h gene was identified as ribosomal protein L3 which is similar to ribosomal protein L7/12, L13P. This show a subunit of ribosomal protein L13P as acetylates from the vgn 0487h gene in *Halobacterium Salinarum.* In the study by Aivaliotis et al. [108] developed a novel method of an industrial enzyme using proteomic tools. Aldehyde dehydrogenase isolated from *Halobacterium salinarum* by recombination technique. The proteome of *Halobacterium salinarum* was analyzed in MALDI-TOF and ESI-TOF

MS/MS with changes in the NaCl concentration and resulted in 14 proteins were importantly down-regulated 3.5M/6M NaCl. The enzyme activity of purified recombinant aldehyde dehydrogenase shown at 1M NaCl.

Gradient resistance of halophilic proteins was quantified using the iTRAQ quantification technique and resulted in 12669 proteins which covering 40 percent of *Halobacterium salinarum*. The Score ratio of protein expression was identified through LC-MS. Less than 6 % of protein expression was moderate (increase & decrease) ratio of each isolate. Totally 14 proteins include decreased proteins expression level of ratio and 537 proteins with the increased protein expression level of proteins among these 29 proteins is similar to IR + isolates. Mn antioxidant of *Halobacterium salinarum* proteins shows maximum tolerance of ionizing radiation which is involved in energy metabolism.

The cytosolic proteome of *Halobacterium salinarum* proteins analyzed and implicated for genome annotation. 2784 proteins coding genes from *Halobacterium salinarum* are derived from the genome sequence. Out of this protein containing 2042 cytosolic protein are separated by 2-D gel electrophoresis. About 611 identified silver stain protein spots are analyzed in MALDI-TOF MS PMF and resulted in 40 % of cytosolic proteins are involved in the development of genome notation and also gene editing assignment.

Membrane soluble cytoplasmic proteins were extracted from *Halobacterium* sp. NRC-1. These proteins were digested using trypsin and analyzed in tandem-mass spectrometry. This protein was predicted using the TMHMM program and resulted in 426 proteins, out of these 232 proteins are soluble fractions and 165 are insoluble fractions, and this protein involved in various metabolic pathways [130]. The large-scale membrane proteins were quantified from *Halobacterium salinarum*. The protein was quantified using 2-D gel electrophoresis and analyzed in mass spectrometry and this quantified 155 membrane proteins and 101 transmembrane proteins domains [131]. N-terminal peptides were extracted from *Halobacterium salinarum* and *Natronomonas pharaonis* using LC-MS/MS. The protein data were derived from genomic sequencing and bioinformatic tools which resulted in large identified N-terminal peptides from archaea & bacteria [132].

7. Haloferax volcanii

Haloferax volcanii is a halophilic archaeon. The proteins from *Haloferax volcanii* were extracted using shotgun proteomics and purified using immobilized metal affinity chromatography. The purified peptide fragments are analyzed in strong cation exchange chromatography/HPLC combined with nano-ESI-QTOF MS/MS using MS data, 1296 proteins are identified through bioinformatics tools. Among 1296 proteins, 32 % of theoretical proteins of *Haloferax volcanii.*

The Stress response phage stock protein PspA in *Haloferax volcanii* under salinity mediated condition. Totally 44 protein spots where detector through LC-MS/MS and their peptide sequence are derived from MS/MS. Most of the proteins are identified under optimal salinity conditions and they are involved in translation transport and metabolism which confirm the protein to study in the highest salinity condition and confirm as a homolog of the PspA protein and play important role *in Haloferax volcanii* and hypersaline adaptation. In the study by Ivanka et al. discussed the development of new 2-D maps of halophilic protein using *Haloferax volcanii* protein through improved 2D gel electrophoresis.

8. *Halomonas* species

The proteomic profile of *Halomonas elongata* is analyzed to find the features of osmoregulation and the resulting 3473 in proteins through Nano LC-MS/MS and bioinformatic analysis. Out of these 470 proteins were increased and decrease in salinity. These 24 genes encode the function of carbohydrate metabolisms such as Glucose degradation, Kreb cycle, ectoine metabolism, and ammonia assimilation. *Halomonas* sp. Strain AAD12 was isolated from the camalti saltern area. This isolate tolerated with phenol and undergoes adaptive changes, these proteins were analyzed through MALDI-TOF MS, and identified proteins involved various metabolic processes [133]. *Halomonas salina* DSMZ 5928 undergoes large-scale proteomic identification. These proteins were analyzed in MALDI- QqTOF MS and identified N-terminal are investigated through bioinformatics tools that resulted in 16 proteins. 11 proteins play an important role in energy metabolism [134].

The moderate halophilic bacteria of *Halomonas elongate* isolated from the soil samples in Iran. Totally 800 protein spots where detector and response to selenite, telluride, and combination of selenite and telluride to the *Halomonas* sp. through 2-D gel electrophoresis. 70 standard protein spots were analyzed in MALDI TOF/TOF MS and resulted in 36 proteins. These include the mechanism of fatty acid synthesis, cell transports, ATP production, oxidative stress, and the bacterial response of DNA replication, translation, and transcription which results in the tolerance or adaptation of *Halomonas* sp. to these two toxic oxyanions.

9. *Haloarcula* species and *halorhodospira* species

Haloarcula maristmortui RR12 was isolated from the solar salt plan and salinity of the strain was analyzed in EDAX with ICP-AES. The proteins from *Haloarcula maristmortui* RR12 were identified using MALDI-TOF MS and identified proteins were response salinity stress adaptation [135]. Proteins from *Halorhodospira halophila* were characterized using isoelectric focusing gel electrophoresis and resultant acidic proteomics were determined using bioinformatics tools [136].

10. *Nocardiopsis* species and *Novosphingobium* species

Nocardiopsis xinjiangenesis halophilic adapting actinobacteria lives in the hypersaline environment. The protein function of *Nocardiopsis xinjiangenesis* is quantified using the Isobaric Tag for Relative and Absolute Quantification (iTRAQ) which identified 685 membrane proteins, out of these 126 membrane proteins are belongs to Salt adaptation which provides protein activity in high salinity stress condition and protects hyperosomotic stress condition. The major membrane protein of outer membrane vesicles (OMVs) was confirmed in *Novosphingobium pentaromativorans* 16 through transmission electron microscopy. The membrane-associated protein fraction characterized using SDS-PAGE and proteins are identified using LC-MS/MS and bioinformatic analysis.

11. Other halophilic bacteria

The Study of halobase, include genomics and proteomics analysis. 23 different halophilic bacteria are analyzed and identified 55,000 genes through genome sequence, 50,000 proteins with 3-D structure through bioinformatic analysis, and also identified 1000 structural RNAs. The three-dimensional structural difference of halophilic and non-halophilic proteins is observed between the osmolyte and mesophilic pairs through data analytic tools. They suggested that the weakening of hydrophobic interactions in salt solution will prevent the loss of functions in high salinity conditions. *Acidihalobacter prosperus* DSM 14174 is an acidophilic halophilic bacterium. The proteome of this organism is to understand the chloride Ion stress tolerance under low pH using SWATH-MS. This study created a chloride tolerance and acidic conditions by *Acidihalobacter prosperus* DSM 14174.

Comparative proteomic analysis of gram-positive and gram-negative of moderate halophilic bacteria provides a preliminary understanding of halophilic protein diversity in different salinity conditions. The gram-positive *Bacillus* sp. EMB9 and *Marinobacter* sp. EMB5 of gram-negative. Halophile's proteome in varying salinity conditions and analyzed in 2-D gel electrophoresis and data analysis and resulted in 235 protein spots from *Bacillus* sp. EMB9 and 83 protein spots from *Marinobacter* sp. EMB5 is detected. In gram-positive bacteria, 12 new protein

Table 2
List of Protein profiling techniques in Halophilic bacteria.

Isolate	Techniques	References
Halobacterium sp. NRC-1	ESI-MS/MS	[130]
Halorhodospira halophila	Isoelectric focusing gel electrophoresis	[136]
Halophilic archaea	2-D gel electrophoresis/MS	[33]
Haloarchaea marismortui RR12	SDS-PAGE/MALDTI-TOF MS	[135]
Halobacterium salinarum	ESI Q-TOF/LC-MS/MS	[137]
Halomonas sp. Strain AAD12	2-D PAGE/MALDI-TOF MS	[132]
Halomonas salina DSMZ 5928	MALDI-TOF MS	[133]
Halobacterium *salinarum*	2-D gel electrophoresis/MS	[134]
Salinicoccus roseus 12	MALDI-TOF MS	[131]

spots, 91 common expression protein spots, and 41 protein spots are suppressed in various salinity conditions. 36 new protein spots, 20 common expressions protein spots, and 27 protein spots are suppressed under low salt concentration.

Salinicoccus roseus W12 isolated from Salt Lake. This isolate was undergone lactate stress response and identified 1656 protein spots treated with sodium lactate and 1843 spots treated with NaCl were detected with 2-D gel electrophoresis and analyzed in mass spectrometry (Table 2). 21 protein spots identified successfully. Out of these 19 protein spots were up-regulated and 2 were down-regulated. These proteins involved in the metabolic and signaling process [131].

12. Conclusion and future perspectives

This review provides the complete coverage of proteomics studies in halophilic bacteria. The techniques are advanced, delicate, and proteomic development in research analysis. Furthermore, the studies as accomplishing in purification, characterization, quantification, and bioinformatic analysis. In future proteomics of halophilic bacteria will provide many uses in biotechnology field like bioremediation, therapeutic drug isolation and biofuels which are yet to be explored. Proteomics in halophilic bacteria has a bright future ahead of it, with numerous important areas where it can make a significant contribution. Such as mechanism of protein – salt tolerance and adaptation in Halophilic bacteria including gene expression studies. Halophilic bacterial proteome has promising wide range of future biotechnological applications especially in medical and pharmaceutical industries such salt-tolerant microbial therapy and probiotic and gut health research. The uses of halophilic bacterial proteomics will grow as proteome methods advance and become more widely available, resulting in novel findings in fields including industrial biotechnology, environmental research, and medicine.

Ethical approval statement

Not applicable.

CRediT authorship contribution statement

Pavithran Kumar: Data curation, Writing – original draft. **Pasiyappazham Ramasamy:** Project administration, Supervision, Writing – review & editing. **Manjunathan Jagadeesan:** Conceptualization, Validation, Visualization.

Declaration of competing interest

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

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Data availability

Data will be made available on request.

References

[1] M. Kaleem Sarwar, I. Azam, T. Iqbal, Biology and applications of halophilic bacteria and archaea: a review, electron, J. Biol. 11 (2015) 98–103.
[2] P. Manikandan, A. Gnanasekaran, S. Pk, Survey of Halophilic Bacterial Diversity from Vedaranyam Salt Pans Area in Nagapattinam District and Applications, 7, 2018, pp. 3394–3397.
[3] J. John, V. Siva, K. Richa, A. Arya, A. Kumar, Life in high salt concentrations with changing environmental conditions: insights from genomic and phenotypic analysis of salinivibrio sp, Microorganisms. 7 (2019), https://doi.org/10.3390/microorganisms7110577.
[4] B.B. Salgaonkar, R. Rodrigues, A study on the halophilic archaeal diversity from the food grade iodised crystal salt from a saltern of India, Microbiol. 88 (2019) 709–719, https://doi.org/10.1134/S002626171906016X. Russian Fed.
[5] S. Rafiq, F. Fathima, S.J. Shahina, K.V. Ramesh, Biodegradation of low density polyethylene (LDPE) by halophilic bacteria isolated from solar saltpans, kovalam, chennai, Nat. Environ. Pollut. Technol. 17 (2018) 1367–1371.
[6] V. Jeyanthi, P. Velusamy, Anti-methicillin resistant Staphylococcus aureus compound isolation from halophilic Bacillus amyloliquefaciens MHB1 and determination of its mode of action using electron microscope and flow cytometry analysis, Indian J. Microbiol. 56 (2016) 148–157, https://doi.org/10.1007/s12088-016-0566-8.
[7] T. Nadu, Biodegradation of hydrocarbons by halophilic bacteria isolated from the saltpans of thoothukudi district, India, Nov, Res. Microbiol. J. 3 (2019) 252–257, https://doi.org/10.21608/nrmj.2019.28111.
[8] I. Print, I. Online, S.R. Ramya, K.R.T. Asha, World Journal of Pharmaceutical Sciences Halophilic Exopolysaccharide Isolated from Swamithope Salt Pans Induce Cell Death Through Apoptosis in A549 , MCF-7 , HT-29 Cancer Cells, 2016.
[9] S. Parthiban, R. Rajasankar, S. Mythili, A. Sathiavelu, Isolation and phylogenetic characterization of extremophiles from marakanam salterns abstract : salterns are highly saline environments, where salt concentrations inhibit growth of Most coll, Ijabpt.Com. (2010) 1279–1284. http://www.ijabpt.com/pdf/83079-Parthiban [1][1].pdf.
[10] Lobna Daoud, Mamdouh Ben Ali, Halophilic microorganisms: interesting group of extremophiles with important applications in biotechnology and environment, in: Physiological and Biotechnological Aspects of Extremophiles, Academic Press, 2020, pp. 51–64, https://doi.org/10.1016/B978-0-12-818322-9.00005-8.
[11] Antonio Ventosa, M. Carmen Márquez, María J. Garabito, David R. Arahal, Moderately halophilic gram-positive bacterial diversity in hypersaline environments, Extremophiles. 2 (1998) 297–304, https://doi.org/10.1007/s007920050072.
[12] Antonio Ventosa, Joaquín J. Nieto, Oren Aharon, Biology of moderately halophilic aerobic bacteria, Microbiol. Mol. Biol. Rev. 62 (2) (1998) 504–544, https://doi.org/10.1128/mmbr.62.2.504-544.1998.
[13] Paulina Corral, Mohammad A. Amoozegar, Antonio Ventosa, Halophiles and their biomolecules: recent advances and future applications in biomedicine, Mar. Drugs. 18 (1) (2019) 33, https://doi.org/10.3390/md18010033.
[14] Alessandro Siglioccolo, Alessandro Paiardini, Maria Piscitelli, Stefano Pascarella, Structural adaptation of extreme halophilic proteins through decrease of conserved hydrophobic contact surface, BMC Struct. Biol. 11 (2011) 1–12, https://doi.org/10.1186/1472-6807-11-50.
[15] Aharon Oren, Novel insights into the diversity of halophilic microorganisms and their functioning in hypersaline ecosystems, Biodiversity. 3 (1) (2024) 18, https://doi.org/10.1038/s44185-024-00050-w.
[16] Aharon Oren, Industrial and environmental applications of halophilic microorganisms, Environ. Technol. 31 (8–9) (2010) 825–834, https://doi.org/10.1080/09593330903370026.
[17] H.R. Karbalaei-Heidari, M.A. Amoozegar, M. Hajighasemi, A.A. Ziaee, A. Ventosa, Production, optimization and purification of a novel extracellular protease from the moderately halophilic bacterium Halobacillus karajensis, J. Ind. Microbiol. Biotechnol. 36 (2009) 21–27, https://doi.org/10.1007/s10295-008-0466-y.
[18] P. Shivanand, G. Jayaraman, Production of extracellular protease from halotolerant bacterium, Bacillus aquimaris strain VITP4 isolated from kumta coast, Process Biochem. 44 (2009) 1088–1094, https://doi.org/10.1016/j.procbio.2009.05.010.
[19] S. Alsaadawi, G. Uslu, Review for environmental treatment applications by halophilic bacteria, Rev. Environ. Treat. Appl. by Halophilic Bact. (2018).
[20] I. Ali, A. Akbar, B. Yanwisetpakdee, S. Prasongsuk, P. Lotrakul, H. Punnapayak, Purification, characterization, and potential of saline waste water remediation of

a polyextremophilic α -amylase from an obligate halophilic aspergillus gracilis, BioMed Res. Int. 2014 (2014), https://doi.org/10.1155/2014/106937.
[21] S. Kumar, S.K. Khare, Chloride activated halophilic α -amylase from marinobacter sp. EMB8: production optimization and nanoimmobilization for efficient starch hydrolysis, Enzym. Res. 2015 (2015), https://doi.org/10.1155/2015/859485.
[22] M.J. Coronado, C. Vargas, J. Hofemeister, A. Ventosa, J.J. Nieto, Production and biochemical characterization of an α-amylase from the moderate halophile Halomonas meridiana, FEMS Microbiol. Lett. 183 (2000) 67–71, https://doi.org/10.1016/S0378-1097(99)00628-X.
[23] X. Li, H.Y. Yu, Purification and characterization of novel organic-solvent-tolerant β-amylase and serine protease from a newly isolated Salimicrobium halophilum strain LY20, FEMS Microbiol. Lett. 329 (2012) 204–211, https://doi.org/10.1111/j.1574-6968.2012.02522.x.
[24] X. Li, H.Y. Yu, Extracellular production of beta-amylase by a halophilic isolate, Halobacillus sp. LY9, J. Ind. Microbiol. Biotechnol. 38 (2011) 1837–1843, https://doi.org/10.1007/s10295-011-0972-1.
[25] A. Elmansy, M.S. Asker, E.M. El-Kady, S.M. Hassanein, F.M. El-Beih, Production and optimization of α-amylase from thermo-halophilic bacteria isolated from different local marine environments, Bull. Natl. Res. Cent. 42 (2018), https://doi.org/10.1186/s42269-018-0033-2.
[26] I.P. Parwata, M. Asyari, R. Hertadi, Organic solvent-stable lipase from moderate halophilic bacteria Pseudomonas stutzeri isolated from the mud crater of Bleduk kuwu, Central Java, Indonesia, J. Pure Appl. Microbiol. 8 (2014) 31–40.
[27] A.H. Mohamedin, A.M. Mowafy, A.A. Elsayed, S.O. Ghanim, Potential applications of some moderate halophilic bacteria, Egypt, J. Aquat. Biol. Fish. 22 (2018) 537–550, https://doi.org/10.21608/ejabf.2019.25337.
[28] J. Biswas, R. Barman, A.K. Paul, Influence of cultural conditions for extracellular lipase production by a halotolerant bacterium, Bacillus sp. Ors4, J. Microbiol. Biotechnol. Food Sci. 7 (2017) 325–331, https://doi.org/10.15414/jmbfs.2017/18.7.3.325-331.
[29] N. Srivastava, M. Srivastava, P.W. Ramteke, P.K. Mishra, Synthetic Biology Strategy for Microbial Cellulases, Elsevier B.V., 2019, https://doi.org/10.1016/b978-0-444-63503-7.00014-0.
[30] M.A. Amoozegar, A. Safarpour, K.A. Noghabi, T. Bakhtiary, A. Ventosa, Halophiles and their vast potential in biofuel production, Front. Microbiol. 10 (2019), https://doi.org/10.3389/fmicb.2019.01895.
[31] B. Bajpai, M. Chaudhary, J. Saxena, Production and characterization of α-amylase from an extremely halophilic archaeon, haloferax sp. HA10, Food Technol. Biotechnol. 53 (2015) 11–17, https://doi.org/10.17113/ftb.53.01.15.3824.
[32] S. Kumar, R. Karan, S. Kapoor, S.P. Singh, S.K. Khare, Screening and isolation of halophilic bacteria producing industrially important enzymes, Braz. J. Microbiol. 43 (2012) 1595–1603, https://doi.org/10.1590/S1517-83822012000400044.
[33] A. Kalwasińska, U. Jankiewicz, T. Felföldi, A. Burkowska-But, M.S. Brzezinska, Alkaline and halophilic protease production by Bacillus luteus H11 and its potential industrial applications, Food Technol. Biotechnol. 56 (2018) 553–561, https://doi.org/10.17113/ftb.56.04.18.5553.
[34] R. Selvarajan, T. Sibanda, M. Tekere, H. Nyoni, S. Meddows-Taylor, Diversity analysis and bioresource characterization of halophilic bacteria isolated from a South African saltpan, Molecules 22 (2017) 1–20, https://doi.org/10.3390/molecules22040657.
[35] P. Shirazian, S. Asad, M.A. Amoozegar, The potential of halophilic and halotolerant bacteria for the production of antineoplastic enzymes: L-asparaginase and L-glutaminase, EXCLI J 15 (2016) 268–279, https://doi.org/10.17179/excli2016-146.
[36] A. Ebrahiminezhad, S. Rasoul-Amini, Y. Ghasemi, l-Asparaginase production by moderate halophilic bacteria isolated from maharloo salt Lake, Indian J. Microbiol. 51 (2011) 307–311, https://doi.org/10.1007/s12088-011-0158-6.
[37] H.Y. Yu, X. Li, Alkali-stable cellulase from a halophilic isolate, gracilibacillus sp. SK1 and its application in lignocellulosic saccharification for ethanol production, Biomass Bioenergy 81 (2015) 19–25, https://doi.org/10.1016/j.biombioe.2015.05.020.
[38] M. T. Sh Al-Rubaye, M.H.J. Al-Musawi, J. Fakhari, M. Hosseini, Screening and characterization of halophilic bacteria with industrial enzymes from salt Lake razazah, Karbala, Iraq, Biosci. Biotechnol. Res. Asia 14 (2017) 531–539, https://doi.org/10.13005/bbra/2476.
[39] W.A. Joo, C.W. Kim, Proteomics of halophilic archaea, J. Chromatogr., B: Anal. Technol. Biomed. Life Sci. 815 (2005) 237–250, https://doi.org/10.1016/j.jchromb.2004.10.041.
[40] Qinghua Xing, Shanshan Zhang, Xinyi Tao, Noha M. Mesbah, Xinwei Mao, Haisheng Wang, Juergen Wiegel, Baisuo Zhao, The polyextremophile Natranaerobius thermophilus adopts a dual adaptive strategy to long-term salinity stress, simultaneously accumulating compatible solutes and K+, Appl. Environ. Microbiol. 90 (5) (2024) e00145 https://doi.org/10.1128/aem.00145-24, 24.
[41] Banaei Esfahani, C. Nicod, R. Aebersold, B.C. Collins, Systems proteomics approaches to study bacterial pathogens: application to Mycobacterium tuberculosis, Curr. Opin. Microbiol. 39 (2017) 64–72, https://doi.org/10.1016/j.mib.2017.09.013.
[42] A. Cupp-Sutton, S. Wu, High-throughput quantitative top-down proteomics, Mol. Omi. 16 (2020) 91–99, https://doi.org/10.1039/c9mo00154a.
[43] C. Villard Calligaris, D. Lafitte, Advances in top-down proteomics for disease biomarker discovery, J. Proteonomics 74 (2011) 920–934, https://doi.org/10.1016/j.jprot.2011.03.030.
[44] A. Becker Tholey, Top-down proteomics for the analysis of proteolytic events - methods, applications and perspectives, Biochim. Biophys. Acta - Mol. Cell Res. 1864 (2017) 2191–2199, https://doi.org/10.1016/j.bbamcr.2017.07.002.
[45] J. Wither, K.C. Hansen, J.A. Reisz, Mass spectrometry-based bottom-up proteomics: sample preparation, LC-MS/MS analysis, and database query strategies, Curr. Protoc. Protein Sci. 2016 (2016), https://doi.org/10.1002/cpps.18, 16.4.1-16.4.20.
[46] D.B. Foster, Manual of Cardiovascular Proteomics (2016), https://doi.org/10.1007/978-3-319-31828-8.
[47] Lopez ferrer, M. Blank, S. Meding, A. Paulus, R. Huguet, R. Swart, A.F.R. Huhmer, Pushing the limits of Bottom-Up proteomics with state-of-the-art capillary UHPLC and orbitrap mass spectrometry for reproducible quantitation of proteomes, Thermo Fish. Sci. (2016) 1–6. https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/AN-639-LC-MS-Bottom-Up-Proteomics-AN64626-EN.pdf.
[48] Albert Bolhuis, Daniel Kwan, Judith R. Thomas, Halophilic adaptations of proteins. Protein Adaptation in Extremophiles: Design, Selection and Applications, Nova Science Publishers, 2008, pp. 71–104.
[49] N. Sharma, M.S. Farooqi, K.K. Chaturvedi, S.B. Lal, M. Grover, A. Rai, P. Pandey, The halophile protein database, Database (2014) bau114, https://doi.org/10.1093/database/bau114.
[50] G. Ortega, T. Diercks, O. Millet, Halophilic protein adaptation results from synergistic residue-ion interactions in the folded and unfolded states, Chem. Biol. (2025) 1597–1607, https://doi.org/10.1016/j.chembiol.2015.10.010.
[51] R. Hahn Jungbauer, Chapter 22 Ion-Exchange Chromatography, first ed., Elsevier Inc., 2009 https://doi.org/10.1016/S0076-6879(09)63022-6.
[52] Fadoua Ghanmi, Alyssa Carré-Mlouka, Zied Zarai, Hafedh Mejdoub, Jean Peduzzi, Sami Maalej, Sylvie Rebuffat, The extremely halophilic archaeon Halobacterium salinarum ETD5 from the solar saltern of Sfax (tunisia) produces multiple halocins, Res. Microbiol. 171 (2) (2020) 80–90, https://doi.org/10.1016/j.ijbiomac.2016.07.001.
[53] Sumit Kumar, Jasneet Grewal, Ayesha Sadaf, R. Hemamalini, Sunil K. Khare, Halophiles as a source of polyextremophilic α-amylase for industrial applications, AIMS Microbiology 2 (1) (2016) 1–26, https://doi.org/10.3934/microbiol.2016.1.1.
[54] Gaurav Sanghvi, Mehul Jivrajani, Nirav Patel, Heta Jivrajani, Govinal Badiger Bhaskara, Shivani Patel, Purification and characterization of haloalkaline, organic solvent stable xylanase from newly isolated halophilic Bacterium-OKH, Int. Sch. Res. Not. (1) (2014) 198251, https://doi.org/10.1155/2014/198251, 2014.
[55] Marjeta Urh, Dan Simpson, Kate Zhao, Affinity chromatography: general methods, Methods Enzymol. 463 (2009) 417–438, https://doi.org/10.1016/S0076-6879(09)63026-3.
[56] Shahla Rezaei, Reza Shahverdi Ahmad, Mohammad Ali Faramarzi, Isolation, one-step affinity purification, and characterization of a polyextremotolerant laccase from the halophilic bacterium Aquisalibacillus elongatus and its application in the delignification of sugar beet pulp, Bioresour. Technol. 230 (2017) 67–75, https://doi.org/10.1016/j.biortech.2017.01.036.
[57] T. Burnouf, M. Radosevich, Affinity chromatography in the industrial purification of plasma proteins for therapeutic use, J. Biochem. Biophys. Methods 49 (2001) 575–586, https://doi.org/10.1016/S0165-022X(01)00221-4.
[58] G. Fassina, M. Ruvo, G. Palombo, A. Verdoliva, M. Marino, Novel ligands for the affinity-chromatographic purification of antibodies, J. Biochem. Biophys. Methods 49 (2001) 481–490, https://doi.org/10.1016/S0165-022X(01)00215-9.
[59] Richard R. Burgess, A brief practical review of size exclusion chromatography: rules of thumb, limitations, and troubleshooting, Protein Expr. Purif. 150 (2018) 81–85, https://doi.org/10.1016/j.pep.2018.05.007.
[60] Clara Barrau, Flaviana Di Lorenzo, Rodolfo Javier Menes, Rosa Lanzetta, Antonio Molinaro, Alba Silipo, The structure of the lipid a from the halophilic bacterium Spiribacter salinus M19-40T, Mar. Drugs 16 (4) (2018) 124, https://doi.org/10.3390/md16040124.
[61] R.M. Lequin, Enzyme immunoassay (EIA)/enzyme-linked immunosorbent assay (ELISA), Clin. Chem. 51 (2005) 2415–2418, https://doi.org/10.1373/clinchem.2005.051532.
[62] Shihuan Zhou, Bochen Pan, Xiaoxue Kuang, Shuhong Chen, Lianghui Liu, Yawen Song, Yuyan Zhao, Xianlin Xu, Xiaoling Cheng, Jiawei Yang, Characterization and mechanism investigation of salt-activated methionine sulfoxide reductase A from halophiles, iScience 27 (9) (2024), https://doi.org/10.1016/j.isci.2024.110806.
[63] D.M. Rissin, C.W. Kan, T.G. Campbell, S.C. Howes, D.R. Fournier, L. Song, T. Piech, P.P. Patel, L. Chang, A.J. Rivnak, E.P. Ferrell, J.D. Randall, G. K. Provuncher, D.R. Walt, D.C. Duffy, Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations, Nat. Biotechnol. 28 (2010) 595–599, https://doi.org/10.1038/nbt.1641.
[64] W. Hogrefe, X. Su, J. Song, R. Ashley, L. Kong, Detection of herpes simplex virus type 2-specific immunoglobulin G antibodies in African sera by using recombinant gG2, Western blotting, and gG2 inhibition, J. Clin. Microbiol. 40 (2002) 3635–3640, https://doi.org/10.1128/JCM.40.10.3635-3640.2002.
[65] Hiroko Tokunaga, Kenjiro Mitsuo, Sachiyo Ichinose, Akira Omori, Antonio Ventosa, Taiji Nakae, Masao Tokunaga, Salt-inducible multidrug efflux pump protein in the moderately halophilic bacterium chromohalobacter sp, Appl. Environ. Microbiol. 70 (8) (2004) 4424–4431, https://doi.org/10.1128/AEM.70.8.4424-4431.2004.
[66] Hiroko Tokunaga, Matsujiro Ishibashi, Tsutomu Arakawa, Masao Tokunaga, Highly efficient renaturation of β-lactamase isolated from moderately halophilic bacteria, FEBS Lett. 558 (1–3) (2004) 7–12, https://doi.org/10.1016/S0014-5793(03)01508-4.
[67] J.B. Smith, Peptide sequencing by edman degradation, encycl, Life Sci. (2001) 1–3, https://doi.org/10.1038/npg.els.0002688.

[68] D.H. Schlesinger, PROTEINS| Traditional Methods of Sequence Determination, 2005, pp. 420–424, https://doi.org/10.1016/B0-12-369397-7/00497-0.

[69] F.X.R. Sutandy, J. Qian, C.S. Chen, H. Zhu, Overview of protein microarrays, Curr. Protoc. Protein Sci. (2013) 1–16, https://doi.org/10.1002/0471140864.ps2701s72.

[70] Satoshi Fukuchi, Kazuaki Yoshimune, Mamoru Wakayama, Mitsuaki Moriguchi, Ken Nishikawa, Unique amino acid composition of proteins in halophilic bacteria, Journal of molecular biology 327 (2) (2003) 347–357, https://doi.org/10.1016/S0022-2836(03)00150-5.

[71] H.J. Issaq, T.D. Veenstra, Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE): advances and perspectives, Biotechniques 44 (2008) 697–700, https://doi.org/10.2144/000112823.

[72] Chang-Won Cho, So-Hee Lee, Jiyeon Choi, Soo-Jin Park, Dong-Jin Ha, Hyo-Jeong Kim, Chan-Wha Kim, Improvement of the two-dimensional gel electrophoresis analysis for the proteome study of Halobacterium salinarum, Proteomics 3 (12) (2003) 2325–2329, https://doi.org/10.1002/pmic.200300525.

[73] Qin De, Feng, Zhang Bo, Lu Wei Dong, Yang Su Sheng, Protein expression analysis of Halobacillus dabanensis $ D-8^ T $ subjected to salt shock, J. Microbiol. 44 (4) (2006) 369–374.

[74] Michael J. Dunn, HM Burghes Arthur, High Resolution two-dimensional polyacrylamide-gel Electrophoresis, Gel Electrophoresis of Proteins, Butterworth-Heinemann, 1986, pp. 203–261, https://doi.org/10.1016/B978-0-7236-0882-0.50009-5.

[75] P. Manikandan, Jamseel Moopantakath, Madangchanok Imchen, Ranjith Kumavath, P.K. SenthilKumar, Identification of multi-potent protein subtilisin A from halophilic bacterium Bacillus firmus VE2, Microb. Pathog. 157 (2021) 105007, https://doi.org/10.1016/j.micpath.2021.105007.

[76] Ankur Das, Mahua Gupta Choudhury, Growth pattern, protein quantification, and Antibiotic responses of four halophilic bacterial strains in different salt concentration, Journal of Microbiology and Infectious Diseases 14 (2) (2024), https://doi.org/10.5455/JMID.2024.v14.i2.2, 46-46.

[77] V. Shakuri, Sahar, Ali Mohammad Latifi, Morteza Mirzaei, Samaneh Khodi, Isolating two native extreme halophilic bacterial strains producing bacteriorhodopsin protein from aran-bidgol Lake, Journal of Applied Biotechnology Reports 3 (3) (2016) 447–451.

[78] Mark Hesselberg, Russell H. Vreeland, Utilization of protein profiles for the characterization of halophilic bacteria, Curr. Microbiol. 31 (1995) 158–162, https://doi.org/10.1007/BF00293547.

[79] M.S. Smyth, J.H.J. Martin, X Ray crystallography, J. Clin. Pathol. - Mol. Pathol. 53 (2000) 8–14, https://doi.org/10.1136/mp.53.1.8.

[80] Laura Matarredona, María José García-Bonete, Jorge Guío, Mónica Camacho, María F. Fillat, Julia Esclapez, María-José Bonete, Global lrp regulator protein from haloferax mediterranei: transcriptional analysis and structural characterization, Int. J. Biol. Macromol. 260 (2024) 129541, https://doi.org/10.1016/j.ijbiomac.2024.129541.

[81] Komal Ghauri, Tjaard Pijning, Nayla Munawar, Hazrat Ali, Muhammad A. Ghauri, Munir A. Anwar, Wallis Russell, Crystal structure of an inulosucrase from Halalkalicoccus jeotgali B3T, a halophilic archaeal strain, FEBS J. 288 (19) (2021) 5723–5736, https://doi.org/10.1111/febs.15843.

[82] S. Wiese, K.A. Reidegeld, H.E. Meyer, B. Warscheid, Protein labeling by iTRAQ: a new tool for quantitative mass spectrometry in proteome research, Proteomics 7 (2007) 340–350, https://doi.org/10.1002/pmic.200600422.

[83] H. Geraili Daronkola, The role of acidic amino acids in the hydration and stabilization of halophilic proteins, Diss. Universität Potsdam (2021), https://doi.org/10.25932/publishup-51671.

[84] T. Mizukami, J.T. Bedford, S. Liao, L.H. Greene, H. Roder, Effects of ionic strength on the folding and stability of SAMP1, a ubiquitin-like halophilic protein, Biophys. J. (2022) 552–564, https://doi.org/10.1016/j.bpj.2022.01.010.

[85] H. Arthanari, K. Takeuchi, A. Dubey, G. Wagner, Emerging solution NMR methods to illuminate the structural and dynamic properties of proteins, Curr. Opin. Struct. Biol. (2019) 294–304, https://doi.org/10.1016/j.sbi.2019.06.005.

[86] N. Gunde-Cimerman, A. Plemenitaš, A. Oren, Strategies of adaptation of microorganisms of the three domains of life to high salt concentrations, FEMS Microbiol. Rev. (2018) 353–375, https://doi.org/10.1093/femsre/fuy009.

[87] S. Biswas, J.M. Rolain, Use of MALDI-TOF mass spectrometry for identification of bacteria that are difficult to culture, J. Microbiol. Methods 92 (2013) 14–24, https://doi.org/10.1016/j.mimet.2012.10.014.

[88] Won-A. Joo, Kim Chan-Wha, Proteomics of halophilic archaea, J. Chromatogr. B 815 (1–2) (2005) 237–250, https://doi.org/10.1016/j.jchromb.2004.10.041.

[89] J.N. Adkins, S.M. Varnum, K.J. Auberry, R.J. Moore, N.H. Angell, R.D. Smith, D. L. Springer, J.G. Pounds, Toward a human blood serum proteome: analysis by multidimensional separation coupled with mass spectrometry, Mol. Cell. Proteomics 1 (2002) 947–955, https://doi.org/10.1074/mcp.M200066-MCP200.

[90] Sheerin Khatib-Shahidi, Malin Andersson, Jennifer L. Herman, Todd A. Gillespie, Richard M. Caprioli, Direct molecular analysis of whole-body animal tissue sections by imaging MALDI mass spectrometry, Anal. Chem. 78 (18) (2006) 6448–6456, https://doi.org/10.1021/ac060788p.

[91] M. Vihinen, Bioinformatics in proteomics, biomol, Eng. 18 (2001) 241–248, https://doi.org/10.1016/S1389-0344(01)00099-5.

[92] B. Vidová, Z. Šramková, L. Tišáková, M. Oravkinová, A. Godány, Bioinformatics analysis of bacteriophage and prophage endolysin domains, Biol 69 (2014) 541–556, https://doi.org/10.2478/s11756-014-0358-8.

[93] Naveen Sharma, Mohammad Samir Farooqi, Krishna Kumar Chaturvedi, Shashi Bhushan Lal, Monendra Grover, Anil Rai, Pankaj Pandey, The halophile protein database, Database 2014 (2014), https://doi.org/10.1093/database/bau114 bau114.

[94] Nina Gunde-Cimerman, Ana Plemenitaš, Aharon Oren, Strategies of adaptation of microorganisms of the three domains of life to high salt concentrations, FEMS Microbiol. Rev. 42 (3) (2018) 353–375, https://doi.org/10.1093/femsre/fuy009.

[95] Guangya Zhang, Lin Yi, Stability of halophilic proteins: from dipeptide attributes to discrimination classifier, Int. J. Biol. Macromol. 53 (2013) 1–6, https://doi.org/10.1016/j.ijbiomac.2012.10.031.

[96] Shantong Hu, Xiaoyu Wang, Zhikang Wang, Menghan Jiang, Shihui Wang, Wenya Wang, Jiangning Song, Guimin Zhang, HPClas: a data-driven approach for identifying halophilic proteins based on catBoost, Mlife 3 (4) (2024) 515–526, https://doi.org/10.1002/mlf2.12125.

[97] S.E. Ong, M. Mann, Identifying and quantifying sites of protein methylation by heavy methyl SILAC, Curr. Protoc. Protein Sci. Chapter 14 (2006) 1–12, https://doi.org/10.1002/0471140864.ps1409s46.

[98] B.M. Zee, R.S. Levin, P.A. Dimaggio, B.A. Garcia, Global turnover of histone post-translational modifications and variants in human cells, Epigenetics Chromatin 3 (2010) 1–11, https://doi.org/10.1186/1756-8935-3-22.

[99] P. Mews, B.M. Zee, S. Liu, G. Donahue, B.A. Garcia, S.L. Berger, Histone methylation has dynamics distinct from those of histone acetylation in cell cycle reentry from quiescence, Mol. Cell Biol. 34 (2014) 3968–3980, https://doi.org/10.1128/mcb.00763-14.

[100] B. Soufi, C. Kumar, F. Gnad, M. Mann, I. Mijakovic, B. MacEk, Stable isotope labeling by amino acids in cell culture (SILAC) applied to quantitative proteomics of bacillus subtilis, J. Proteome Res. 9 (2010) 3638–3646, https://doi.org/10.1021/pr100150w.

[101] Ricardo L. Couto-Rodríguez, Jin Koh, Sixue Chen, Julie A. Maupin-Furlow, Insights into the lysine acetylome of the Haloarchaeon Haloferax volcanii during oxidative stress by quantitative SILAC-based proteomics, Antioxidants 12 (6) (2023) 1203, https://doi.org/10.3390/antiox12061203.

[102] A. Westman-Brinkmalm, A. Abramsson, J. Pannee, C. Gang, M.K. Gustavsson, M. von Otter, K. Blennow, G. Brinkmalm, H. Heumann, H. Zetterberg, SILAC zebrafish for quantitative analysis of protein turnover and tissue regeneration, J. Proteonomics 75 (2011) 425–434, https://doi.org/10.1016/j.jprot.2011.08.008.

[103] U. Distler, J. Kuharev, P. Navarro, S. Tenzer, Label-free quantification in ion mobility-enhanced data-independent acquisition proteomics, Nat. Protoc. 11 (2016) 795–812, https://doi.org/10.1038/nprot.2016.042.

[104] L.N. Mueller, M.Y. Brusniak, D.R. Mani, R. Aebersold, An assessment of software solutions for the analysis of mass spectrometry based quantitative proteomics data, J. Proteome Res. 7 (2008) 51–61, https://doi.org/10.1021/pr700758r.

[105] K.A. Neilson, N.A. Ali, S. Muralidharan, M. Mirzaei, M. Mariani, G. Assadourian, A. Lee, S.C. Van Sluyter, P.A. Haynes, Less label, more free: approaches in label-free quantitative mass spectrometry, Proteomics 11 (2011) 535–553, https://doi.org/10.1002/pmic.201000553.

[106] Chuanfa Liu, Guanting Niu, Xiaowen Li, Huchen Zhang, Huawei Chen, Dongxia Hou, Ping Lan, Zhi Hong, Comparative label-free quantitative proteomics analysis reveals the essential roles of N-glycans in salt tolerance by modulating protein abundance in arabidopsis, Front. Plant Sci. 12 (2021) 646425, https://doi.org/10.3389/fpls.2021.646425.

[107] Y. Shiio, R. Aebersold, Quantitative proteome analysis using isotope-coded affinity tags and mass spectrometry, Nat. Protoc. 1 (2006) 139–145, https://doi.org/10.1038/nprot.2006.22.

[108] Andreas Tebbe, Alexander Schmidt, Kosta Konstantinidis, Michaela Falb, Birgit Bisle, Christian Klein, Michalis Aivaliotis, et al., Life-style changes of a halophilic archaeon analyzed by quantitative proteomics, Proteomics 9 (15) (2009) 3843–3855, https://doi.org/10.1002/pmic.200800944.

[109] T.M. Pawlik, D.H. Hawke, Y. Liu, S. Krishnamurthy, H. Fritsche, K.K. Hunt, H. M. Kuerer, Proteomic analysis of nipple aspirate fluid from women with early-stage breast cancer using isotope-coded affinity tags and tandem mass spectrometry reveals differential expression of vitamin D binding protein, BMC Cancer 6 (2006) 1–10, https://doi.org/10.1186/1471-2407-6-68.

[110] Mo Li, Qianqian Wang, Xuefei Song, Jingjing Guo, Junrui Wu, Rina Wu, iTRAQ-based proteomic analysis of responses of Lactobacillus plantarum FS5-5 to salt tolerance, Ann. Microbiol. 69 (2019) 377–394, https://doi.org/10.1007/s13213-018-1425-0.

[111] Stefan Leuko, Mark J. Raftery, Brendan P. Burns, Malcolm R. Walter, Brett A. Neilan, Global protein-level responses of Halobacterium salinarum NRC-1 to prolonged changes in external sodium chloride concentrations, J. Proteome Res. 8 (5) (2009) 2218–2225, https://doi.org/10.1021/pr800663c.

[112] John Jumper, Richard Evans, Alexander Pritzel, Tim Green, Michael Figurnov, Olaf Ronneberger, Kathryn Tunyasuvunakool, et al., Highly accurate protein structure prediction with AlphaFold, nature 596 (7873) (2021) 583–589, https://doi.org/10.1038/s41586-021-03819-2.

[113] Michael C. Thompson, O. Yeates Todd, Jose A. Rodriguez, Advances in methods for atomic resolution macromolecular structure determination, F1000Research 9 (2020), https://doi.org/10.12688/f1000research.25097.1. F1000-Faculty.

[114] Anisha Debnath, Aveepsa Sengupta, Sujata Rudrapal, Ashutosh Kumar, Mamta Rani, In-silico study of molecular adaptations in halophilic Cas9, Lett. Appl. Microbiol. 78 (2) (2025), https://doi.org/10.1093/lambio/ovaf006 ovaf006.

[115] M.E. Barrios-Llerena, P.K. Chong, C.S. Gan, A.P.L. Snijders, K.F. Reardon, P. C. Wright, Shotgun proteomics of Cyanobacteria - applications of experimental and data-mining techniques, briefings funct, Genom. Proteonomics 5 (2006) 121–132, https://doi.org/10.1093/bfgp/ell021.

[116] D.M. Tracz, S.J. McCorrister, P.M. Chong, D.M. Lee, C.R. Corbett, G. R. Westmacott, A simple shotgun proteomics method for rapid bacterial identification, J. Microbiol. Methods 94 (2013) 54–57, https://doi.org/10.1016/j.mimet.2013.04.008.
[117] A.C. Vergunst, D. O'Callaghan, Host-bacteria interactions, Methods Mol. Biol. 1197 (2014) 1197, https://doi.org/10.1007/978-1-4939-1261-2.
[118] X. Yao, A. Freas, J. Ramirez, P.A. Demirev, C. Fenselau, Proteolytic 18O labeling for comparative proteomics: model studies with two serotypes of adenovirus, Anal. Chem. 73 (2001) 2836–2842, https://doi.org/10.1021/ac001404c.
[119] R.J. Beynon, J.M. Pratt, Metabolic labeling of proteins for proteomics, Mol. Cell. Proteomics 4 (2005) 857–872, https://doi.org/10.1074/mcp.R400010-MCP200.
[120] E.S. Boja, H.M. Fales, Overalkylation of a protein digest with iodoacetamide, Anal. Chem. 73 (2001) 3576–3582, https://doi.org/10.1021/ac0103423.
[121] S. Suttapitugsakul, H. Xiao, J. Smeekens, R. Wu, Evaluation and optimization of reduction and alkylation methods to maximize peptide identification with MS-based proteomics, Mol. Biosyst. 13 (2017) 2574–2582, https://doi.org/10.1039/c7mb00393e.
[122] L. Tsiatsiani, A.J.R. Heck, Proteomics beyond trypsin, FEBS J. 282 (2015) 2612–2626, https://doi.org/10.1111/febs.13287.
[123] M. Widbiller, H. Schweikl, A. Bruckmann, A. Rosendahl, E. Hochmuth, S. R. Lindner, W. Buchalla, K.M. Galler, Shotgun proteomics of human dentin with different prefractionation methods, Sci. Rep. 9 (2019) 1–8, https://doi.org/10.1038/s41598-019-41144-x.
[124] H.V. Kilambi, K. Manda, H. Sanivarapu, V.K. Maurya, R. Sharma, Y. Sreelakshmi, Shotgun proteomics of tomato fruits: evaluation, optimization and validation of sample preparation methods and mass spectrometric parameters, Front. Plant Sci. 7 (2016) 1–14, https://doi.org/10.3389/fpls.2016.00969.
[125] S.K. Swanson, M.P. Washburn, The continuing evolution of shotgun proteomics, Drug Discov. Today 10 (2005) 719–725, https://doi.org/10.1016/S1359-6446(05)03450-1.
[126] E.I. Chen, J.R. Yates, Cancer proteomics by quantitative shotgun proteomics, Mol. Oncol. 1 (2007) 144–159, https://doi.org/10.1016/j.molonc.2007.05.001.
[127] Y.V. Karpievitch, A.D. Polpitiya, G.A. Anderson, R.D. Smith, A.R. Dabney, Liquid chromatography mass spectrometry-based proteomics: biological and technological aspects, Ann. Appl. Stat. 4 (2010) 1797–1823, https://doi.org/10.1214/10-AOAS341.
[128] C. Warinner, J. Hendy, C. Speller, E. Cappellini, R. Fischer, C. Trachsel, J. Arneborg, N. Lynnerup, O.E. Craig, D.M. Swallow, A. Fotakis, R.J. Christensen, J.V. Olsen, A. Liebert, N. Montalva, S. Fiddyment, S. Charlton, M. MacKie, A. Canci, A. Bouwman, F. Rühli, M.T.P. Gilbert, M.J. Collins, Direct evidence of milk consumption from ancient human dental calculus, Sci. Rep. 4 (2014) 1–6, https://doi.org/10.1038/srep07104.
[129] K. Jung, Statistical analysis in proteomics, Methods Mol. Biol. 1362 (2016) 313, https://doi.org/10.1007/978-1-4939-3106-4.
[130] Y.A. Goo, E.C. Yi, W.A. Tao, N.S. Baliga, M. Pan, D.R. Goodlett, L. Hood, W.V. Ng, Proteomic analysis of an extreme halophilic archaeon halobacterium species NRC-1, Proc. 50th ASMS Conf. Mass Spectrom. Allied Top (2002) 221–222.
[131] H. Wang, L. Wang, H. Yang, Y. Cai, L. Sun, Y. Xue, B. Yu, Y. Ma, Comparative proteomic insights into the lactate responses of halophilic Salinicoccus roseus W12, Sci. Rep. 5 (2015) 1–11, https://doi.org/10.1038/srep13776.
[132] S. Ceylan, B.S. Akbulut, A.A. Denizci, D. Kazan, Proteomic insight into phenolic adaptation of a moderately halophilic halomonas sp. strain AAD12, Can. J. Microbiol. 57 (2011) 295–302, https://doi.org/10.1139/w11-009.
[133] Salman Dilgimen, Aydan, Proteomics Studies of Halomonas salina and a New Moderately Halophilic Bacteria Living in Saltern Areas, Diss, 2008.
[134] B. Bisle, A. Schmidt, B. Scheibe, C. Klein, A. Tebbe, J. Kellermann, F. Siedler, F. Pfeiffer, F. Lottspeich, D. Oesterhelt, Quantitative profiling of the membrane proteome in halophilic archaeon, Mol. Cell. Proteomics 5 (2006) 1543–1558, https://doi.org/10.1074/mcp.M600106-MCP200.
[135] R.S. Thombre, V.D. Shinde, R.S. Oke, S.K. Dhar, Y.S. Shouche, Biology and survival of extremely halophilic archaeon Haloarcula marismortui RR12 isolated from mumbai salterns, India in response to salinity stress, Sci. Rep. 6 (2016) 6–15, https://doi.org/10.1038/srep25642.
[136] R. Deole, J. Challacombe, D.W. Raiford, W.D. Hoff, An extremely halophilic proteobacterium combines a highly acidic proteome with a low cytoplasmic potassium content, J. Biol. Chem. 288 (2013) 581–588, https://doi.org/10.1074/jbc.M112.420505.
[137] M. Aivaliotis, K. Gevaert, M. Falb, A. Tebbe, K. Konstantinidis, B. Bisle, C. Klein, L. Martens, A. Staes, E. Timmerman, J. Van Damme, F. Siedler, F. Pfeiffer, J. Vandekerckhove, D. Oesterhelt, Large-scale identification of N-terminal peptides in the halophilic archaea Halobacterium salinarum and Natronomonas pharaonis, J. Proteome Res. 6 (2007) 2195–2204, https://doi.org/10.1021/pr0700347.