JOURNAL OF TROPICAL LIFE SCIENCE
2021, Vol. 11, No. 3, 339 – 360
http://dx.doi.org/10.11594/jtls.11.03.11

Review Article

A Review on Enzymatic Response of Bacteria to Salt Stress and Genomic/Metagenomic Analysis of Adaptation Protein in Hypersaline Environment

Habeebat Adekilekun Oyewusi [1, 2, 3*], Muhammad Muhammad [1, 4], Roswanira Abdul Wahab [3, 5], Fahrul Huyop [1, 3]

[1] Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia.
[2] Department of Biochemistry, School of Science and Computer Studies, Federal Polytechnic Ado Ekiti, PMB 5351, Ekiti State, Nigeria.
[3] Enzyme Technology and Green Synthesis Group, Faculty of Science, Universiti Teknologi Malaysia, 81310, UTM Johor Bahru, Johor, Malaysia.
[4] Department of Microbiology and Biotechnology, Federal University Dutse, PMB 7156, Jigawa State Nigeria.
[5] Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, 81310, UTM Johor Bahru, Johor, Malaysia.

Article history:
Submission April 2021
Revised April 2021
Accepted June 2021

**Corresponding author:*
E-mail: habbyfat@gmail.com

ABSTRACT

Halophilic bacteria adapted to high salinity (low water activity) provide insight into how they naturally maintain efficient cell integrity under high osmotic stress. Almost all microbes adapt to extreme situations either by intracellular amass inorganic ions (K^+) to counterbalance high salt concentration or by synthesizing and accumulating certain organic solutes called compatible solutes that confer protection without affecting cell functions. The use of a culture-independent method like genomic or metagenomics shields more light on the microbial diversity, gene structure and regulation, and discovery of novel genes that led to understanding their adaptation mechanism and roles in extreme environments. Therefore, microbes that survive this natural attenuation aimed at acclimatizing with the extreme environments could serve as the sources of biotechnologically essential molecules with an extensive array of uses. This review provides an overview of adaptation strategy of bacteria in hypersaline environments revealed by genomic and metagenomic studies.

Keywords: Enzymatic response, Genomic analysis, Hypersaline environments, Metagenomic, Salt stress

Introduction

Microbes living in extreme habitats are categorized by strong physico-chemical properties such as salinity, temperature, pH and radiation. Their extreme environments signify an interesting source of bacteria variety and metabolic activities with exciting biotechnological capability [1, 2]. Microbes inhabiting such environments are called extremophiles, which show physiological and metabolic adaptations to their environmental situations. For example, they possess enzymes, which allow them to adapt to extreme situations [3, 4]. The marine/hypersaline habitat is regarded as mostly unexploited source for the detection of a novel kinds of enzymes/genes. Moreover, emphasis on the huge diversity of bacteria occurs on earth's seas and oceans, which may produce novel kinds of biomolecules [4-7]. These biomolecules provide vital roles to the generating microbes and their neighbours, including animals and plants. Likewise, they have broader consequences to the marine habitats and world biogeochemical cycles and climate changes [5, 8]. Alternatively, owing to

How to cite:
Oyewusi HA, Muhammad M, Wahab RA, Huyop F (2021) A review on Enzymatic Response of Bacteria to Salt Stress and Genomic/Metagenomic Analysis of Adaptation Protein in Hypersaline Environment. Journal of Tropical Life Science 11 (3): 339 – 360. doi: 10.11594/jtls.11.03.11.

the osmoadaptation ability and metabolic capability to produce compatible solutes, nutritional products, bioplastics, biopolymers, and halophilic enzymes, they signify substantial agents in numerous industries such as chemicals and the environment biofuel, bioremediation, pharmaceutical and health care [6, 9, 10].

The saline environment forms one of the most fundamental properties of the marine realm. Many factors influence the levels of marine salinity, including water inflow to the river, rainfall, wind and melting glaciers and evaporation. Salinity is a crucial environmental factor of microbial community structure and dispersion, this exerts a high evolutionary selective force [11, 12]. The changes in salinity symbolize the environmental barrier, which is difficult for microbes to cross over [13,14]. The cause could be primarily due to the osmoregulation costs of the energy involved and the necessity of precise adaptive mechanisms in dealing with high-salt concentrations [15, 16]. The effects of salinity are detrimental on the microbes when it is not neutralized. Cells in hypertonic conditions stimulate the osmotic gradient that reduces the comparative cell water content, thus leaves a highly concentrated cytoplasm behind. In this situation, failure to adapt to these conditions results in aggregation, precipitation and denaturation of nucleic acids, proteins and other biomolecules, thereby disrupting their structure and functions [17-20]. Consequently, enzymes lose their flexibility and catalytic activity [21, 22]. Nevertheless, thriving communities of microbes capable of withstanding such conditions are found in many hypersaline surroundings [7, 23-25] due to the recent awareness of the ecological importance of microbes in hypersaline systems [9, 26-28]. However, the in-depth investigation of a wide range of halo-adaptation mechanisms provided substantial information about halo-adaptation strategies originated from prokaryotic studies. Therefore, the review discussed two different approaches emanated from evolution to address osmotic stress.

The enzymatic response/adaptation mechanisms used by halophiles to familiarize themselves with high-salt stress are categorised into two. The first one is salt-in cytoplasm strategies, which are common to microbes that can regulate their cell turgor pressure with aim to prevent any possible water loss. Secondly, microbes can also retort to high-salt conditions by using a compatible solute strategy that entails intracellular synthesis or uptake of compatible solutes from neighbouring surroundings to stabilize the cytoplasmic substance with the outside [29-32]. Genomic is the analysis of the sequence, structural and genome content, genes and their number, functions and organisation along the genome. However, metagenomic sequencing discovers the potential in microbial communities and offers insights into the diversity, life cycle and function of numerous microbial communities. For instance, functional metagenomics is the analysis of the gene function and the control of their expression at the organ or cell level and organism by decoding the dynamics of gene transcription, translate and protein-protein interactions on a genome-wide scale using high-throughput technologies [33-35]. Understanding the salt adaptation approaches by employing the genome sequencing method is confined to certain extreme environments. Although halophiles share certain common halo-adaptive characteristics, the disparities in their genetic variety indicate numerous molecular pathways for halo-adaptation and a huge diversity in adaptive approaches [18,31]. Therefore, the currents review looks at an overall adaptation strategy of the microbes in the hypersaline environment and how these properties/strategies correlate to the analysis of metagenomic and genomic functional studies.

Adaptation of halophiles to marine/hypersaline environments

Halotolerance refers to when the living organisms adapt to the high salinity conditions. High-salt environments influence protein stability, solubility and subsequent function [36]. Therefore, the dehydration of cellular proteins becomes noticeable as a result of reduced water substance in the cells due to the water-molecules entrapped in external ionic latices [37]. This interaction that upsets internal microbial proteins triggered by dehydration might similarly be prevented by modulating their net charge. The greater percentage of glutamate and aspartate on the surfaces differentiate halophilic proteins from non-halophilic proteins [38].

There are two key characteristics processed by halophiles that allowed them to adapt to extreme salinity. The first one is the negativity of halophile's proteomes. This possesses many negative charges and improved hydrophobicity, which are accountable for the molecular haloadap-tation of halophilic enzymes [38, 39]. Secondly, the occur-

rence of the multiple copies of signal transducer and transcriptional regulators might be relevant in their haloadaption. The halophilic genomes have combined structures consisting of an acidic proteome, multiple copies of general transcriptional factors and multiple replicons, including a high G-C content [40]. These are beneficial for avoiding UV-induced injures that cause the formation of thymine dimer and dangerous mutations [41]. However, the combined feature is not common, and it is obvious that the G+C-bias might be a rare feature of halophiles because the general G-C contents of some halophiles are scarcely below 55% [30, 42, 43]. Therefore, this adaptation sets obvious constraints on the surround during which these microbes will occur since the proteins constantly need a pretty high intracellular salt concentration for appropriate protein folding and activity [44, 45]. Halophilic microorganisms typically embrace either compatible solute strate-gy or salt-in strategy to survive in the saline envi-ronments [46].

'Salt-in cytoplasm' strategy

Salt-in cytoplasm strategy adaptation defends halophiles in a saline environment where they amass inorganic ions intracellularly to correct the effect of high salt concentration. This approach process includes chloride channels or pumps that transport chloride ions from the environment into the cytoplasm and the channel has arginines and/or lysines to aid chloride uptake and discharge [47]. Some halophilic bacteria accrue potassium chloride (KCl) to molar concentrations with aim of neutralizing the outside salinity [48, 49]. However, the mechanism of KCl accretion is well understood in some halophiles by using electrical potential, which runs the potassium ion uptake in the halophiles results due to the intensive effort of the membrane-bound proton-pump bacteriorhodopsin, the "proton gradient-consuming" proteins ATP synthase and Na^+/H^+ antiporter [49, 50]. Potassium is absorbed through a potassium ion uniport mechanism. For better absorption, the electrical potential of the transport must be higher than the diffusion potential of potassium ion. The counterion chloride is either absorbed by primary or secondary transporters such as Na^+/H^+ antiporter, which is replaced by Cl^-/Na^+ symporter in the absence of light [49,50]. Therefore, halophiles have an extremely adapted, salt-tolerant system or maybe salt-dependent enzyme equipment, which indicates a high volume of acidic and low quantity of aromatic acid sequences compared to non-halophiles [49, 51].

Up to date, the biochemical analysis for K^+ and Cl^- uptake in most halophiles like *Salinibacter ruber* had not been established. *S. ruber* maintains its osmotic balance by accumulating K^+ in an extreme-salt environment [52]. The genome analysis of these bacteria suggests that K^+ maybe absorbed via Tropomyosin receptor kinase HA (*TrtHA*) transport mechanism. The bacteria genome encodes four copies of known trkA, a cytoplasmic membrane surface protein that binds NADH/ NAD+ and is crucial for transport activity. The two copies of the known trkH sequence encode a membrane spanning translocated subunit that originates from unrelated sources through lateral gene transfer [53]. Furthermore, *S. ruber* imports chloride ion via the chloride pump called halorhodopsin and the other four putative genes encode rhodopsin were recognized. However, based on sequence identity, two of these genes encode sensory rhodopsin, the third gene encodes a proton pump while the fourth one encodes a chloride pump. Remarkably, two copies of genes encoding Na^-K/Cl cotransporter that play a vital role in accumulating potassium and chloride ions are found in eukaryotes but not in prokaryotes. However, *S. ruber* as a member of prokaryotes possesses these two copies of gene [53]. The physical properties of bacterial on how they are adapted to hypersaline environments had been vividly explained by genomic or metagenomic studies.

'Compatible solute' strategy

Microbial integrity is threatened by changing the microbial host's osmotic pressure, including the danger of cell lysis owing to unexpected downshift in outside salinity and a quick parallel inflow of water. Reacting to this condition, it is possible to expel the compatible solutes from the cell quickly. However, with the increase in time, they could be metabolised into other forms, which does not serve the purpose of osmolyte [54]. Compatible solutes as an effective microbial mechanism can withstand both extreme and fluctuating salinities (Figure 1). Compatible solutes are defined as tiny, low-molecular-weight organic molecules that are soluble in water up to a molar concentration and do not interfere with cell metabolism [55].

They are classified into two chemical classes. The first group belongs to the amino acids; their derivatives include glutamate, glutamine, proline, ectoine, glycine alkaloid and N-acetyl-β-lysine [56]. The second group belongs to the sugars and polyols families, they include trehalose, glycerol, dimyo-inositol-1,1' phosphate (DIP) and sulfotrehalose [57]. These compatible solutes are often accumulated with the accomplishment of medium uptake or by *de novo* synthesis.

The general adaptation of microbes using compatible solutes is depicted in Figure 1. Compatible solutes such as glutamate and glutamine are accumulated after low or moderate increases in salinities. However, glycine betaine uptake is favoured over *de novo* synthesis of other compatible solutes in the presence of glycine betaine (Figure 1) [58]. With a further increase in salinity, the glutamate pool hits a critical threshold value, which effectively activates the proline genes' transcription, leading to proline synthesis. At higher salinities (2.0-3.0 M NaCl), this event triggers proline accumulation as dominant compatible solute (Figure 1). As a result of this signal, the expression of ectoine genes can then be induced. Although proline may be the signal, a physiological scenario must be imagined that results in a loss of proline and increased ectoine. It is worth noting that the synthesis or uptake of compatible solutes may be a chloride-dependent process either directly or indirectly.

Major compatible solutes in halophiles: Biosynthesis, uptake, and their regulation

Halophiles like *Halobacillus halophilus* deal with effect of external salt by gathering the cocktail of different compatible solutes primarily amino acids such as glutamate, glutamine, proline and the amino acid derivatives like glycine betaine, ectoine, N^{ε}-acetyl lysine and N^{δ}-acetyl ornithine [55].

Glutamate and glutamine biosynthesis

The action of three key enzymes synthesizes glutamate and glutamine. Glutamate biosynthesis can be achieved via the aid of glutamate dehydrogenase (GDH) or glutamate synthase (GOGAT) while glutamine is produced with the aid of glutamine synthetase. The gene orientation of these enzymes is shown in Figure 2. Investigation on the genome sequence analysis of *H. halophilus* revealed the presence of two recognized open reading frame (orf) coding for glutamate dehydrogenase (*gdh1* and *gdh2*) (Figure 2a). While genome analysis revealed one additional orf encoding large subunit of a glutamate synthase (*gltA*) in addition to two orf potentially encoding the small subunit of a glutamate synthase (*gltB1* and *gltB2*) [59] (Figure 2b). When *H. halophilus* cells were exposed to sudden increase in the salinity, the genes encoding the subunits for the glutamine synthetase were not expressed, however, one of the recognized glutamate dehydrogenase gene (*gdh1*) was expressed and there was increase in mRNA level. This established that glutamate production as compatible solute in *H. halophilus* is via the catalytic activity of a glutamate dehydrogenase, which translates one molecule of 2-oxoglutarate and one molecule of ammonium to one molecule of glutamate. The expression of glutamine synthe-

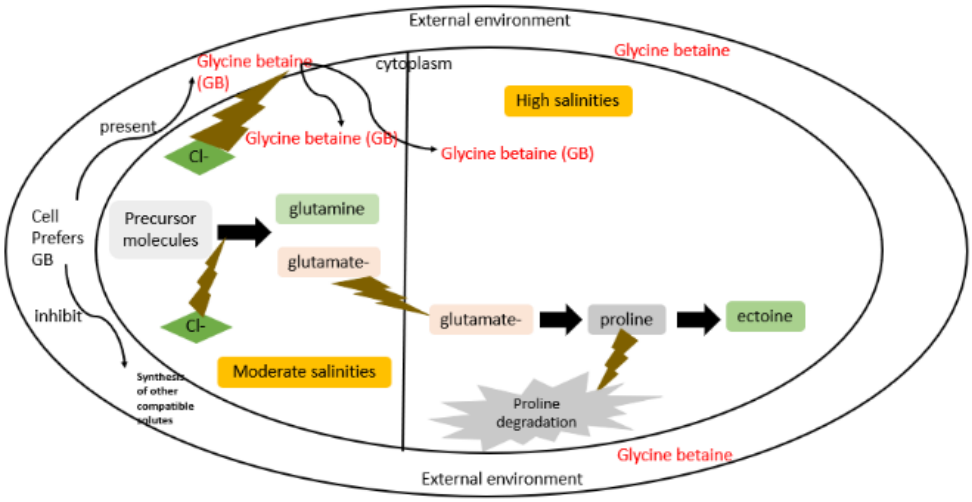

Figure 1. Adaptation mechanism of halophiles using compatible solutes strategies [55]

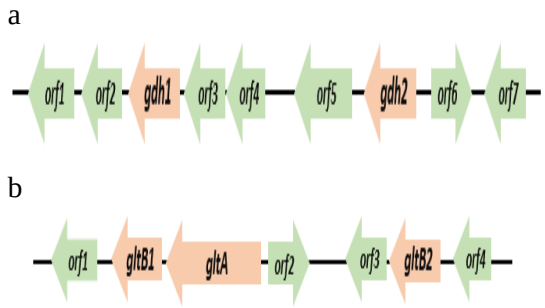

Figure 2. Genetic organization of genes in the biosynthesis of glutamine and glutamate in *Halobacillus halophilus***:** (a) Glutamate dehydrogenase (*gdh*): in the ORFs, a putative glutamate dehydrogenase (*gdh1* and *gdh2*) was identified. In addition, *orf*1, *orf2, orf3, orf4 and orf6* encoded L-asparaginase, pyridine nucleotide-disulfide oxidoreductase family protein (thioredoxin reductase), D-alanyl-D-alanine ligase A, a negative regulator of genetic competence and enoyl-[acyl-carrier protein] reductase respectively, while the *orf5* and *orf7* remained unidentified (b) Glutamate synthase (*glt*) was a heterodimer: large subunit and small subunit were encoded as *gltA* and *gltB* respectively. In *H. halophilus* genome, only one ORF was identified as GltA and two ORFs were identified as GltB labelled as *gltB1* and *gltB2*. Catalase, L-aminopeptidase/D-esterase, dihydropyrimidine dehydrogenase and β-alanine synthase were identified as *orf1, orf2, orf3 and orf4*, respectively [55].

Figure 3. The proline operon of *H. halophilus* possessed three genes encoding a pyrroline-5-carboxylate reductase (*proH*), a glutamate 5-kinase (*proJ*) and a glutamate 5 semialdehyde dehydrogenase (*proA*) [55].

tase was observed at moderate not at unexpectedly increased salinity. This observation provides information about the importance of glutamine and glutamate as a precursor molecule for other compatible solutes like proline and ectoine at extreme salinity.

Proline biosynthesis

Halophilic bacteria such as *H. halophilus* amassed proline in response to an increase in salinity and are prominent over the glutamate and glutamine as the main compatible solutes at higher salinity [60]. The analysis of the genome showed a cluster of three genes, which are a gene encodes a recognized pyrroline-5-carboxylate reductase (*ProH*), a recognized glutamate 5-kinase (*ProJ*) and a recognized glutamate 5-semialdehyde dehydrogenase (*ProA*), which are structured in an operon that encodes for recognized proline biosynthesis enzymes (Figure 3). Therefore, the three enzymes were enough to synthesis proline from glutamate. The bacteria would respond to an increase in salinity by immediately increasing the ProHJA mRNA level, thereby increasing the proline concentration at six hours. This revealed that salinity activates proline production. Curious-ly, the biosynthesis of proline could also be activated by increase in glutamate concentration which significantly enhanced the concentration of *ProHJA* mRNA. The inspiring consequence of glutamate on the transcription of the *ProHJA* genes was subsequently titrated and revealed that a negligible amount of glutamate (0.2 M) is enough to excite the proline gene transcription [60]. Consequently, both sodium chloride and the internal glutamate concentration are accountable for primary signal that activates the proline biosynthesis with increasing salinity. Hence, glutamate is considered as “second messenger” in *H. halophilus* aside as compatible solute.

Ectoine biosynthesis

In most of halophiles, ectoine is not dominated at elevated salinity but proline is produced. However, in contrary to *H. halophilus*, ectoine is mainly produced at elevated salinities [55]. *ectABC* which represent biosynthetic genes of ectoine from aspartate semialdehyde were recognized and represented to produce an operon (Figure 4). *EctA* encodes putative diaminobutyric acid and catalyzes acetylation of L-2,4 diaminobutyrate to CoA and N^{γ}-acetyl-L-2,4-diaminobutyrate. *EctB* encodes putative diaminobutyrate-2-oxoglutarate transaminase and catalyzes the first transamination reaction of L-aspartate-β-semialdehyde to L-2,4 diaminobutyrate. *EctC* encodes putative ectoine synthase and serves as the main enzyme in ectoine production, it catalyzes the intramolecular ring closure of N^{γ}-acetyl-L-2,4-diaminobutyrate to ectoine. Some organisms can convert ectoine to hydroxyectoine, which is another potent compatible solute [61]. To carry this conversion, it requires an evolutionarily conserved hydroxylase

Figure 4. The ectoine operon of *H. halophilus* which entails three diffrent genes encoding a diaminobutyric acid acetyltransferase (*ectA*), a diaminobutyrate-2-oxoglutarate transaminase (*ectB*) and an ectoine synthase (*ectC*) [55].

that belongs to a non-haem, comprising iron (II)- and 2-oxoglutarate-dependent dioxygenase superfamily [62]. The mRNA level of *ectABC* genes was drastically increased when the cell was placed in elevated salinities. Therefore, ectoine synthesis was regarded as one of the osmoregulation. Determining the concentrations of the solute during the growth differentiates proline function from that of ectoine. It was noticed that proline concentrations were drastically reduced when cells attained the stationary phase while ectoine concentrations raised and became dominant in the late stationary growth phase.

Glycine betaine uptake and biosynthesis

Glycine betaine, a trimethylated derivative of the amino acid glycine, it is the most broadly used compatible solute in nature [63]. Microbes could utilize various ways to boost the concentration of glycine betaine intracellularly. Glycine betaine can be synthesized or sourced from the environment externally. The precursor of biosynthesis of glycine betaine is usually from amino acid glycine or choline. However, choline obtained from exogenous sources is more energetically preferable to its *de novo* synthesis. ATP-binding cassette (*ABC*) or secondary transporters are used to uptake compatible solutes in many microbes. Research had recognized the presence of ABC transporter (*ProU*) and the proton motive force-driven known as high-affinity uptake system (*BetT*) in some bacteria such as *E. coli*. *BetT* conveys choline at low concentrations while *ProU* takes it up at higher concentrations [64]. However, the two strongly related ABC transport systems with high-affinity (*OpuB* and *OpuC*) were detected in *Bacillus subtilis* [65]. The *OpuC* conveys other compatible solutes while *OpuB* is specific for choline uptake [66]. Genes encoding enzymes like choline dehydrogenase (*cdh*) or choline oxidase (*cox*) contributed to synthesizing glycine betaine, usually from choline. They speed-up the choline conversion to obtain betaine aldehyde. Betaine aldehyde dehydrogenase is the NAD^+-dependent enzyme that catalyzes betaine aldehyde to glycine betaine. Microorganisms can produce glycine betaine from glycine through a stepwise methylation of amino acid glycine [67-69]. Methylation reactions consist of three steps, they are catalyzed by glycine sarcosine methyltransferase (*Gsm*), which catalyses the methylation of glycine or sarcosine to produce sarcosine or dimethylglycine and sarcosine dimethylglycine methyltransferase (*Sdm*) catalyses the methylation of sarcosine or dimethylglycine to dimethylglycine or glycine betaine, respectively.

Trehalose uptake and biosynthesis

Trehalose is a non-reducing disaccharide, produced and exploited extensively by microbes as compatible solute. It provides and protects osmostress against low- and high-temperature stress. In addition, it preserves the functionality of macromolecules and cells under dehydration stress [70]. The disaccharides sucrose and trehalose defend cells from environmental stress [71-73]. Intracellular rise in trehalose had been described in bacteria when treated in sodium chloride [74, 75]. Some bacteria transferred sugars to the cell through the phosphoenolpyruvate-dependent sugar phosphotransferase system (PTS) that conveys and phosphorylates the sugar [76, 77]. Based on structural resemblances, PTS IIBC subunit sugar transport genes were also detected in many bacteria. It is a putative gene involved for the transport and metabolism of trehalose [78,79]. Three genes *treP*, *treA* and *treR* involved in uptake, metabolism & regulation of trehalose [78, 79]. The *treP* gene encodes putative trehalose permease that phosphorylates and translocates trehalose inform of trehalose 6-phosphate across membrane which is subsequently hydrolyzed by *treA* gene encoding phosphos-α-1,1-glucosidase to generate glucose 6-phosphate and glucose [79, 80]. *treR* gene encodes transcriptional regulator, which controls the uptake and metabolism of trehalose [80, 81]. *treP* and *treA* are arranged as operon which is adversely controlled at transcriptional level by trehalose repressor *treR*, possessed a N-terminal α-helix turn-helix sequence domain characteristic of DNA-binding proteins [82, 83] (Figure 5). Based on similarities in amino acid sequence, *treR* is categorized as one of the *FadR-GntR* transcriptional regulatory protein family; it

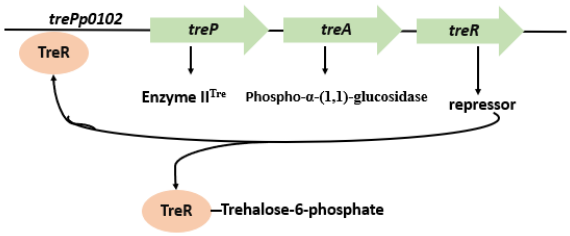

Figure 5. Genetic organization of the *tre* operon. It contains of *treP, tre A* and *tre R* is located downstream [80].

has not been properly studied [82]. The promoter region of *tre* operon based on sequence analysis had shown two palindromes associate with similar sequence to hypothetical operator structures, which is recognized by the *FadR-GntR* transcriptional regulatory protein family [82].

Microbes produce trehalose via various biosynthentic pathways, the route is most famous distributed one and catalyzed by *OtsAB* enzymes [84]. In these pathways, glucose-6-phosphate acts as a precursor and is transformed into trehalose-6-phosphate by α, α-trehalose-phosphate synthase (*OtsA*). Afterward, the intermediate is dephosphorylated by trehalose-6-phosphate phosphatase (*OtsB*) to produce free trehalose [70].

Role of chloride ion in regulating compatible solutes uptake and synthesis

In numerous halophiles, high organic anions concentration requires counter ions such as potassium and/or sodium ions. Nevertheless, some halophiles solely use inorganic anions for osmotic balance. The most prevalent inorganic anion in halophiles is chloride ion and the molar concentrations have been detected in many halophilic archaea. This anion is co-transported with sodium ion or impelled into the cells by halorhodopsin. This anion contributes to osmotic balance, it seems to have influential roles in haloadaptation [85]. For example, the regulation of betaine transportation by chloride has been described. Little is recognized about other inorganic anion compositions of halophiles, aside from chloride.

Furthermore, chloride-dependent signal transduction chain does not essentially require the passage of chloride into the cell. Nevertheless, it can include membrane-bound sensors. In some halophilic microbes, the role of the chloride-dependent regulatory network is indispensable to their growth. The *H. halophilus* growth is severely chloride-dependent. However, one critical role of moderate halophilic microbes is sensing the external salinity and responding to its proceeding transcriptional, translational and enzymatic activity level to regulate the intracellular pool level of the compatible solutes. However, the nature of the signal recognized remains unknown, it was hypothesised that chloride is employed as a signal molecule for external salinity in *H. halophilus* [86]. Thus, this is validated by the discovery that the transport of glycine betaine is sternly chloride dependent. Additionally, there was impairment of glycine betaine in the absence of chloride after osmotic upshock but reinstated by addition of chloride [86]. The other members of bacteria domain, which maintain and require high intracellular chloride concentrations are *H. praevalens* and *S. ruber* [87]. In all the three microbes, the amount of chloride required to adapt in high salt stress had been demonstrated. In many cases, chloride is required to activate specific enzymes as shown in Figure 6.

The ability of a moderate halophile to tolerate an extensive variety of salinities depends on its evolutionary success. However, moderate halophiles such as *H. halophilus* amasses compatible solutes to preserve its turgor under hyperosmotic settings. The biosynthetic pathways and genes for the major solutes as well as their control had been recognized in the evolving genome sequence (Figure 6). *H. halophilus* possessed an exciting shift in its osmolyte approach in salinities within the range of 0.8 to 2 M of sodium chloride. At low salinities, the glutamate and glutamine are the main solute while proline dominates at higher salinities [60]. Additionally, it changes from proline to ectoine synthesis at high salinities and stationary growth phase [55]. The forming of N-acetyl-lysine and N-acetyl-ornithine occurs in trivial volumes, which could drive glycine betaine from the medium [58]. It does not produce glycine betaine *de novo* but can take up choline from the medium and oxidise it to glycine betaine through glycine betaine aldehyde (Figure 6) [88]. Furthermore, to increase the salt concentrations stimulate glycine betaine uptake from the medium requires both sodium and chloride ion. Chloride ion is also directly involved in regulating compatible solutes synthesis (Figure 6). Glutamate and glutamine are synthesized by glutamate synthase and glutamine synthetase, respectively and this activity in whole cells of *H.*

enzymes and compounds from uncultured bacte-

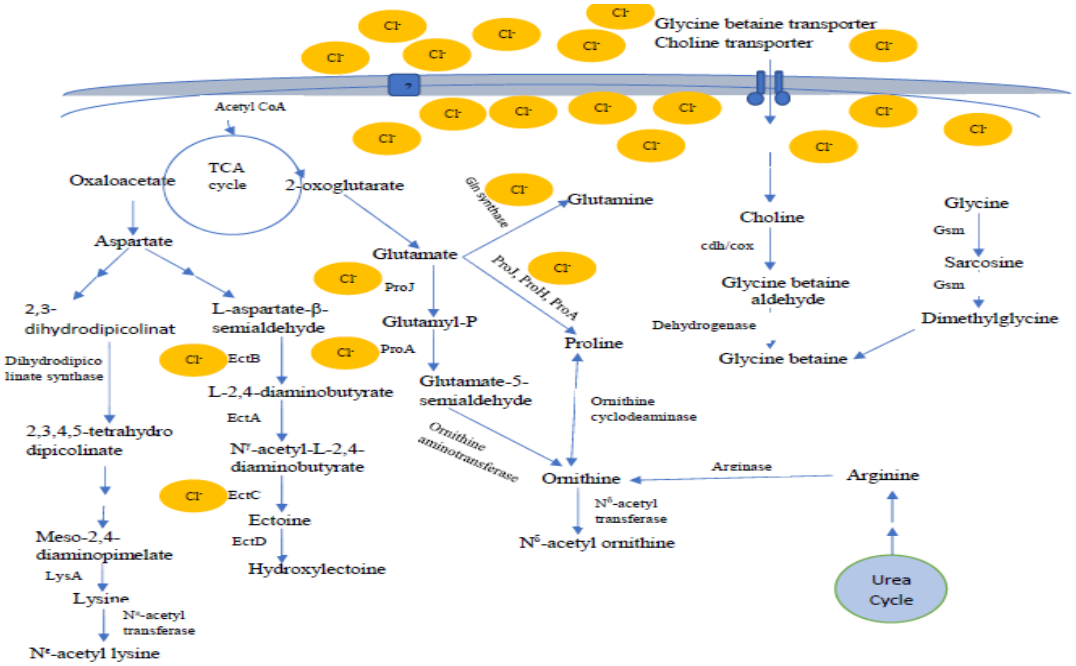

Figure 6. Uptake and biosynthesis of compatible solutes regulated by chloride ion [55, 89]

halophilus is strictly chloride ion dependent [59, 89]. Interestingly, this activity does elucidate the chloride dependence of glutamine and glutamate production and that of proline produced using glutamate as a precursor. Moreover, the expression of proline biosynthetic genes is stimulated by glutamate [55, 90]. Consequently, appraisal of molar concentrations of compatible solutes collected with molar intracellular chloride concentrations introduces the hypothesis that *H. halophilus* uses combined compatible solutes and chloride salts to survive the salt stress. This relatively exceptional trait signifies a transitional process in the evolution of salt adaptation [55].

Microbial metagenomics

It was previously reported that more than 99% of microbes are presently problematic or cannot be merely cultivated in the laboratory [91], which implies our understanding of microbes is essentially restricted in less than 1% of microbes [92]. Those uncultivated microbes own fantastic usage ability, and their metabolites can generate quantities of useful compounds. In addition, the deficiency is taken care by the advent and improvement of metagenomic and this aids in exploring and provides better understanding of the potential micro-world. The widely recognition of metagenomics suggests its utilization in discovering the novelty in genes, ria. Many novelties had been achieved in naming functional genes as well as active substances through the knowledge of metagenomics, which provides extensive application in many fields. It has also been used to ascertain and recognize the novelty in natural genes and compounds in environmental samples through sequence-based screening and function-based screening techniques. The sequence-based screening is used by PCR technology in detecting the gene and compounds existence. In contrast, function-based screening is carried out by examining the activity or expression of enzymes or genes [34, 69, 93-95]. These stated two technologies possessed the decoded amounts of novel genes, which encode vital biocatalysts from uncultured bacteria living in several certain surroundings. Metagenomic sequencing entails examining a sample of the ecosystem without cultivating individual colonies. Metagenomic sequencing is classified as either amplicon or taxonomical metagenomic and shotgun metagenomic, based on the literature.

Taxonomical metagenomic, known as amplicon or targeted metagenomic technique, uses microbial marker genes such as 16S rRNA, internal transcribed spacer and other marker genes. This approach had been recently expanded our ability to infer the functional contribution of individual bacteria community members by mapping

a subset of abundant 16S rRNA sequences to their nearest sequenced reference genome [6, 7]. Presently, using computational tools such as PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) to predict microbial functions from 16S rRNA gene sequencing data is a common alternative to shotgun metagenomic approaches. Meanwhile, shotgun metagenomic sequencing will uncover potential microbial communities and provide insights into their diversity, life cycle, and functions. Gene fragment recruitment, *de novo* gene prediction, and protein family classification are all options for functional annotation in shotgun metagenomic reads encoding genes of interest. This method answers two important questions about microbial ecology: "who is there" and "what are they doing". The first step of functional annotation is the assignment of biological functions and the second step is the discovery of genes in the data that form biological networks, such as metabolic pathways. Several studies had been performed and led to the promising discovery of complementary metabolic pathways in microbes that make up a community.

A rapid and technological innovation of high-throughput sequencing and genomics techniques such as metagenomics, large-scale microbial community analysis is enabled, and this becomes a competent method to investigate the ability of particular microbes in various environments. This serves as another method to rRNA sequencing in investigating the complex microbial community structure and allowing the screening of a functional and helpful potential of the environments [96-98]. The greener sequencing tools had paved way for subfield of metagenomics like metatranscriptomics and metaproteomic. The advance in molecular methods and bioinformatics had ensured the sequencing of amassed genomic DNA and numerous challenges related to the marine microbial investigation. Therefore, the whole era of metagenomics sequencing had aided the in-depth thought in indicating a wide range of species in various environments [99-101]. Since assembly is not essential for transcript analysis, several metagenomic studies had used 454 sequencing technology to produce enough length reads, permitting the functional predictions established on a single read, which were subsequently processed gene-centrically [102-104]. However, the general functions' predictions can be made and therefore, phylogenetic assignments for specific functional genes remained impossible most of the time.

Microbial diversity analysis of extreme environments is crucial because of their varied and diverse ecology that can assist in solving the fundamental mechanisms of their adaptation and their role in generating vital compatible solutes that of biotechnologically essential. Metagenomics had presented novel understandings not only on the microbial community composition but also on the novel genes and metabolic activities accountable for microbial adaptation of microbes residing in the environments, such as discovering compatible solutes. The significance of natural microbes is famous, but very few findings detail their adaptation to environmental changes, such as increase or decrease salinity was studied [105, 106]. Comprehensive analysis of varied environments and their adaptation to environmental changes can be well-recognized with the help of metagenomics analysis.

The biodiversity of several hypersaline environments was surveyed using metagenomic sequencing, but little information is on halophilic bacterium capable of retaining cell integrity via accumulation of compatible solutes (Table 1). Sequences associated with genes of compatible solutes were mostly glutamate synthase, betaine transporters, glycerol kinase, glycerol-3 phosphate dehydrogenase, choline dehydrogenase and trehalose synthase while glycerol and glutamate transporters were at lower frequencies. By employing rigorously scheduled assembly methods such as *de novo* or reference assembly, novel microbes or enzymes will be uncovered in metagenomic data. Very little is thought regarding the mechanisms of osmoadaptation by microbes flourishing in hypersaline environments.

The enormous diversity and unculturable nature of certain microbes make it highly complicated to accurately depict microbial communities in a specific ecological niche. Metagenomics is an innovative idea exploring microbial biodiversity, their adaptation to the ecological niches and their evolution [99, 100, 107]. Metagenomic datasets are acquired by high-throughput sequencing of environmental samples and offer an aggregation of the bottlenecks related with conventional molecular techniques of recovering genetic information for a specific environment. High throughput bioinformation assessment allows the precise disco-

Table 1. Metagenomic analysis of microbial communities and adaptation proteins in hypersaline environments

Sample location	Salinity (%)	% Abundant Taxa		Adaptation properties	Approach used	References
		Taxonomic Phylum	*Taxonomic Genus/Order*	Compatible Solute		
Santa Pola Salterns Alicante (Spain)	13	Euryarchaeota, γ-Proteobacteria, α- Proteobacteria, Actinobacteria, Bacteroidetes, Unclassified bacteria, Verrucomicrobia, β-Proteobacteria,	*Spiribacter, Oceanicola, Roseovarius, Aquiluna. Pseudoruegeria, Gracilimonas Puniceicoccus, Alkalilimnicola, Rhodoluna, Agrococcus, Arhodomonas, Haloquadratum*	More abundant sequences related to glutamate biosynthesis and glycerol transport, followed by glycerol biosynthesis and betaine transport. In addition, only few sequences related to glutamate transport, choline transport, and trehalose biosynthesis were found in both (13% and 19%) salinity gradient. Also, large number of sequences related to trehalose transport and glycerol transport were found.	Shotgun metagenomic	[109]
	19	Euryarchaeota, γ-Proteobacteria, α-Proteobacteria, ε-Proteobacteria, Bacteroidetes, Unclassified bacteria, Firmicutes, Actinobacteria, δ-Proteobacteria, Verrucomicrobia,	*Halorubrum, Haloquadratum, Alkalilimnicola, Salinibacter, Natronomonas, Leifsonia Halomicrobium, Clavibacter, Nitrococcus, Arhodomonas, Puniceicoccus, Roseovarius, Aquiluna, Renibacterium, Renibacterium, Halobacterium Haloferax, Pseudoruegeria, Roseobacter, Gramella, Natronobacterium, Planktoluna, Dinoroseobacter, Salicibacter, Flavobacterium, Psychoflexus*		Shotgun metagenomic	[110]
	33	Euryarchaeota, Bacteroidetes, Unclassified bacteria, Archaea,	*Haloquadratum, Halorubrum, Natronomonas, Salinibacter, Haloplanus*.	Detection of numerous sequences associated with choline dehydrogenase, glutamate synthase and trehalose synthase. In addition, they possessed many sequences associated with glycerol kinase, glycerol-3-phosphate and dihydroacetone kinase.	Shotgun metagenomic	[111]
	37	Euryarchaeota, Bacteroidetes, ε-Proteobacteria, Unclassified archaea,	*Haloquadratum, Salinibacter, Halorubrum, Halobacterium, Halorhabdus, Haloferax, Salosimplex, Halobacterium*		Shotgun metagenomic	[110]
Isla Cristina Salterns. (IC21) Huelva Southwest Spain	21	Euryarchaeota, Bacteroidetes, γ-Proteobacteria, Other bacteria.	*Halorubrum, Psychoflexus, Natronomonas, Haloquadratum, Spiribacter*, Unclassified/other bacteria.	Little sequences associated with choline dehydrogenase, glutamate synthase, trehalose synthase was identified. In addition, they possessed many sequences associated with	Shotgun metagenomic	[112]

Continues…

Sample location	Salinity (%)	% Abundant Taxa		Adaptation properties	Approach used	References
		Taxonomic Phylum	*Taxonomic Genus/Order*	Compatible Solute		
				glycerol kinase, glycerol-3-phosphate and dihydroacetone kinase.		
Lake Tyrrell Victoria Australia	24-35	Euryarchaeota Nanoarchaeota Unclassified/other bacteria	*Haloquadratum, Halonotius, Nonohaloarchaea, Halorhabdus,* *Halobaculum, Halorubrum,* Unclassified/other bacteria.	ND	Taxonomic or amplicon metagenomic	[113]
Maras Salterns Peruvian Andes	25-31	Haloarchaea Bacteroidetes γ-Proteobacteria α-Proteobacteria Firmicutes	*Haloquadratum, Halobacterium* *Pseudomonas, Haloarcula* *Halorubrum, Marinococcus* *Halogeometriculum* *Rhodospirillum, Salinibacter*	ND	Taxonomic or amplicon metagenomic	[114]
Majorca Saltern Island Spain	35	Bacteroidetes γ-Proteobacteria α-Proteobacteria Firmicutes β-Proteobacteria Verrucomicrobia β-Proteobacteria Cyanobacteria	*Rhodobacterales, Rhizobiales* *Rhodospirilalles, Salinibacter* *Pseudomonadales, Bacillales* *Alteromonadales, Chromatiales* *Desulfovibrionales,* *Desulfuromonadales,* *Clostridiales,*	ND	Taxonomic or amplicon metagenomic	[115]
Guerrero Negro Saltern. Mexico	18-38	Haloarchaeon Bacteroidetes α-Proteobacteria γ-Proteobacteria δ-Proteobacteria β-Proteobacteria	*Haloquadratum, Halobacterium* *Haloarcula, Halorubrum* *Halorhabdus, Natronococcus* *Halobiforma, Methanococcus* *Microbacterium, Salinibacter* *Puniceicoccus, Spiribacter* *Sediminibacterium*	ND	Taxonomic or amplicon metagenomic	[116]
Shark Bay Western Australia	>60PSU	α-Proteobacteria, γ-Proteobacteria, ε-Proteobacteria, Actinobacter, Cyanobacteria, Bacteroidetes, Planctomycetes, Unclassified bacteria.	*Halococcus, Halobacterium* *Natrialba, Haloarcula* *Natronomonas, Halorubrum* *Halogenometricum, Haloferax* *Methanohalobium, nanoarchacum*	Most sequence genes with choline and betaine uptake, betaine biosynthesis and genes encoding pathways involving in trehalose biosynthesis were observed	Shotgun metagenomic	[117]
Organic lake. Vestfold hills, East Antartical	20	α-Proteobacteria γ-Proteobacteria δ-Proteobacteria ε-Proteobacteria Flavobacteria Acinobacteria	*Marinobacter, Psychromonas* *Halomonas, Saccharospirillum* *Alteromonadales, Roseovariou* *Albinomas, Loktanella* *Rhodobacteriales, Psychroflexus* *Brumimicrobium, Arcobacter* *Flavobacteriales, Desulfotignum* *Desulfophila, Bdellovibrionales* *Peredibacter Bacteriovorax*	ND	Taxonomic or amplicon metagenomic	[118]

Continues…

Sample location	Salinity (%)	% Abundant Taxa		Adaptation properties	Approach used	References
		Taxonomic Phylum	*Taxonomic Genus/Order*	Compatible Solute		
			Desulfosalsimonas Aquiluna Desulfobacterium, Demequina Desulfuromonas, Micrococcales Sulfurimonas, Sulfurospirillum			
Deep-sea lake Thetis	34.8	α-Proteobacteria γ-Proteobacteria β-Proteobacteria ε-Proteobacteria δ-Proteobacteria Bacteroidetes Planctomycetes Cyanobacteria Actinobacteria Deferribacters archaea		ND	Taxonomic or amplicon metagenomic	[119]
Deep-sea lake Medee. Northern Europe	30.4-34.5	α-Proteobacteria γ-Proteobacteria δ-Proteobacteria Bacteroidetes Planctomycetes Verrucomicrobia Acidobacteria Nitrospirae Firmicutes Actinobacteria Chloroflexi Prochlorococcus Archaea		Glycine-betaine uptake genes were observed	Shotgun metagenomic	[120]
Odiel Saltmarshes Southwest Spain	24.0d S/m	Euryarchaeota, Balneolaeota, Rhodothermaeota, γ-Proteobacteria, Bacteroidetes.	*Halorubrum, Fodinibius, Halolamina, Salinigranum, Unclassified Halobacteria, Salinibacter, Gracilimonas, Halohasta, Halopricum, Halobellus, Natronomonas, Halonotius, Haloplanus, Halorientalis, Halorubellus, Halomicroarcula, Halomonas Salinimicrobium,* Unclassified *Ectothiorhodospiraceae,* Unclassified *γ-Proteobacteria, Halalkalicoccus.*	Genes related to trehalose biosynthesis, betaine synthesis from choline and uptake were detected. Genes related to ectoine synthesis and transport were the least abundant of the analysed osmolytes.	Shotgun metagenomic	[100]
	54.4d S/m	Euryarchaeota, Bacteroidetes, γ-Proteobacteria, Balneolaeota, α-Proteobacteria, Actinobacteria.	*Salinimicrobium, Salinigranum, Halolamina, Haloarcula,* Unclassified *Halobacteria, Halobellus, Gracilimonas,* Unclassified *γ-Proteobacteria, Halorubrum, Halomicroarcula, Marinobacter, Nitronomonas, Pseudidomarina, Halomarina Altererythrobacter, Haloplanus, Halorientalis, Fodinibius, Halomonas, Nocardiopsis,*		Shotgun metagenomic	[100]

Continues…

Continues…

Sample location	Salinity (%)	% Abundant Taxa		Adaptation properties	Approach used	References
		Taxonomic Phylum	*Taxonomic Genus/Order*	Compatible Solute		
			Unclassified *Flavobcteriaceae, Idiomarina.*			
Deep-sea hypersaline anoxic Basins (DHABs) (both the upper and lower layer)	7-27.6	Euryarchaeota Thaumarchaeota α-Proteobacteria β-Proteobacteria γ-Proteobacteria ε-Proteobacteria δ-Proteobacteria Bacteroidetes Planctomycetes Firmicutes Spirachaecae.	*Halobacteria, Methanobacteria* *Methanococci, Thermococci* *Methanomicrobia, Desulfuromonadales* *Mycococcales, Desulfobacterales, Chlostridia* *Bacillus, Deferribacterales* *Megnetococcales* *Pseudonomadales*	Expression of glutamate synthase increase as the salinity increase. Other genes for proline biosynthesis such as pyroline-5-carboxylate reductase, glutamate-5-kinase, glutamate-5-semialdehyde dehalogenase and choline dehydrogenase which are the key enzyme for glycine betaine biosynthesis showed a similar pattern. Choline-glycine betaine transporter and trehalose-6-phosphate synthase were also in abundant in the upper layer than lower layer	Shotgun metagenomic	[121]
Great Salt Lake, Utah	5-27	Euryarchaeota Korarchaeota Crenarchaeota Nanoarchaeota Proteobacteria Actinobacteria Bacteroidetes/Chlorobi Deinococcus thermus Aquificae, Firmicutes, Cyanobacteria, Spirochaetes, Chlamydiae,	*Natronomonas, Haloarhabdus,* *Halorubrum, Haloquadratum* *Haloferax, Halogeometricum,* *Haloarcular, Halobacterium,* *Shewanella, Halomonas, Idiomarina, Alcanivorax,* *Puedomonas, Marinobacter*	Genes encoding in the synthesis and uptake of compatible solutes such as betaine aldehyde dehydrogenases and betaine/carnitine/choline transporters were observed.	Amplicon and Shotgun metagenomic	[122]
Dead sea (western part of Jordan)	34	Proteobacteria Firmicutes Bacteroidetes Actinobacteria Cyanobacteria Euryarchaeota Other Archaea	*Acinetobacter, Bacillus, Pseudomonas, Haloarhabdus,* *Natronomonas, Haloplanus* *Halobellus, Halorubrum* *Halobacterium, Halogranum* *Halomicrobium*	ND	Taxonomic or amplicon metagenomic	[123]
Lake Tuz (in Turkish, Tuz	32.4	Firmicutes Proteobacteria Fusobacteria	*Clostridium sensu stricto 1, Clostridium sensu stricto 7, Preptoniphillus, Ramboutsia*	Genes involving in the synthesis and uptake of	Taxonomic or amplicon	[7]

Continues…

Sample location	Salinity (%)	% Abundant Taxa		Adaptation properties	Approach used	References
		Taxonomic Phylum	*Taxonomic Genus/Order*	Compatible Solute		
Gölü) (central plateau of Turkey)		Acidobacteria Bacteroidetes Actinobacteria Cyanobacteria Deinococcus thermus	*Paraclostridium, Lactococcus, Streptococcus, Vagococcus, Enterococcus, Macrococcus, Lysinibacillus, Cetobacterium, Vibrio,Nautella Photobacterium, Proteus, Klebsiella, Escherichia-shigella, Bacillus, Turicibacter, Basea Leucobacter, Aquibacter, Peudochrobacterium, Meiothermus, Shingomonas, Btadyrhizobium, Shewanella, Ralstonia.*	compatible solutes such as ectoine, glycine-betaine, glutamate, trehalose and choline were predicted or observed.	meta-genomic	

very of a gene of interest [100, 108]. Comparative analysis of various ecosystem and their adaptation to environmental changes can be well-recognized by the benefit of the metagenomic analysis, which provide information about the genes responsible for adaptation in such environments.

A vast task in metagenomics studies have been established. The technique depends on taking a community as a whole and overlooks the perspective of individual species. Functional profile of each community can be generated by allocating a read to a specific functional group, assisting in assessing the communities consistent with their functional profiles [124-126]. This method produces an excellent finding with singleton sequencing reads, although its resolution falls when it begins dealing with functional gene annotation. In metagenomics database, deficient annotation of biochemical pathways may occur, resulting to the annotation of metagenomic sequencings by referring to these databases being incredibly challenging. Though, specific enrichment strategies can be used to gain understanding into the genomes of the minor or major members of the community. This method would allow us to achieve functional perceptions into the community as a whole and into specific community members, resulting in a fulfilled and significant sequence data analysis and a high-resolution information on functional genomics. Therefore, complete genome sequencing of bacterial could offer a clear summary of genes organization and their regulation. So far, few studies on the adaptation of various microbes inhabiting hypersaline environments using either or both strategies of adaptation revealed by complete genome sequencing have been reported.

Microbial genomics

Table 2 summarises the adaptation strategies and ascertain important biosynthesis and transport genes from bacterial isolated from hypersaline environments. These studies are useful to comprehend how organisms endure and adapt to the high osmolality environments. For example, the whole genome sequencing of *H. smyrnensis* AAD6T reported the adaptation strategy associated with the uptake of choline betaine, biosynthesis of glycine betaine (GB), ectoine and hydroxyectoine [9]. *H. smyrnensis* AAD6T has two distinct GB/PB high-affinity uptake systems, OpuA and ProU. OpuA system belongs to members of the ABC transporter superfamily and consists of OpuAA, OpuAB and OpuAC, which encodes membrane-associated ATPase, intracellular membrane protein, and extracellular ligand-binding protein. The extracellular OpuAC proteins binds glycine-betaine (GB) or proline-betaine (PB) with high affinity and deliver it to the OpuAA/OpuAB protein complex for the release of substrate into the cytosol with an ATP-dependent substrate translocation mechanism. However, ProU which is a second ABC transporter system imports the GB/PB into the cytoplasm. ProU protein system consists of a GB/PB binding protein (ProX) and permease protein (ProW and ProV). ProU protein binds GB/PB and translocate them through ProWV permease protein complex into the cytosol (Figure 6a). Additionally, GB can also be produced from choline which involved high affinity choline uptake protein BetT, choline dehydrogenase (BetA) and be

Table 2. Whole genome analysis of adaptation proteins/genes of bacterial isolated from marine/hypersaline environments

Locations	Salinity (%)	Microbial species	Adaptation genes	References
Çamaltı Saltern Area Turkey	3-25	*Halomonas smyrnensis* AAD6T	The genome encoding *ectABCD*, ABC transporter systems: *OpuA* and *ProU* genes and *betI* shows that the bacterial can both synthesis and uptake compatible solutes ectoine and glycine betaine.	[9]
Isla Cristina Salterns. Huelva Southwest Spain	13-40	*Spiribacter salinus* M19-40^T *Spiribacter* sp. UAH-SP71	The presence of ABC-type glycine betaine transporters (*betABHI)*, ABC transporters *OpuA* and *ProU*, *TeaABC*-type TRAP transporter, *ectABC* and trehalose synthesis genes (*OtsAB)* predicts the synthesis and uptake of compatible solutes glycine betaine, ectoine, hydroxylectoine and trehalose. Also, the genome has *TrK*-type K^+ uptake systems (*TrkG* and *TrkH),* genes related to K^+ efflux system (*kefC*-type) and a multi-component Mrp Na^+ extrusion system, all these genes show that bacterial can also adapt using "salt in" strategy.	[127-129]
Deep-sea hypersaline anoxic Basins (DHABs), Eastern Mediterranean	7-27.6	*Virgibacillus dokdonensis* strain 21D	The presence of glycine betaine transporter (*OpuD*), glycine betaine/carnitine/choline ABC transporter (*OpuC*) and glycine betaine/carnitine ABC transporter (*Gbu*) predict glycine betaine uptake. The presence of *ectABC* shows ectoine synthesis.	[32]
Arctic marine Zhanjiang Guangdong Province, China	3.5 and above	*Mesonia* sp. HUA40, *Mesonia* algae K4-1	The genome of bacterial glycine betaine transporter (*OpuD*), choline/glycine/valine betaine transporter (*BetT*) involve in glycine betaine uptake. Na^+/H^+ transporter genes used to adapt extreme salinity using "salt in" strategy.	[33]
Marakkanan salt pan, South India	1.9-4.5	*Salinivibrio* sp. HTSP	The presence of *betB, betL* and *betT, ectABC* genes and glycerol uptake facilitator (*GlpF*) indicates that the bacterial uptake or synthesis the compatible solutes glycine betaine, ectoine and glycerol. Genes involved in "salt in" strategies are also predicted like K^+ uptake systems (*TrkA* and *TrkH),* K^+ efflux systems (*kqt, kefB,* and *kefG),* K^+ homeostasis genes (*AATP, FkaB, TAPb, FkaA, KtrA* and *KtrB)* and Na^+ transport systems (H^+/Cl^-ClcA and Na^+/H^+ antiporter).	[130]
Lake Magadi, Eastern African Rift Valley, Kenya	>10	*Cyanobacterium euhalothece* sp. Z-M001	The presence of monovalent cation transporters (Na^+/H^+ antiporters), multi-subunit Na^+/H^+ antiporters (*MrpA-G),* K^+ transporters (*KtrA/B* and *KdpA-D),* K^+ uptake protein (*TrkA*) are used by the bacterial to adapt to high salinity. Compatible solutes genes involved in biosynthesis of trehalose (*treS and treY),* and proline (*ProA/B/C)* are predicted from the genome analysis.	[35]

a

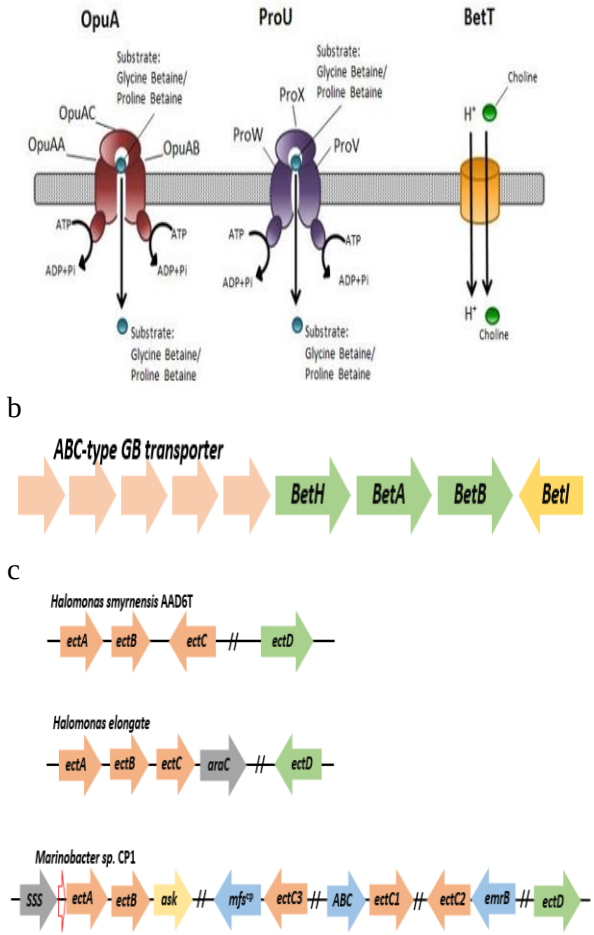

Figure 6. (a) The suggested orientation of Glycine Betaine/Proline Betaine and Choline uptake system in *H. smyrnensis* AAD6T (b) Genetic orientation of glycine betaine genes (c) Genetic orientation of ectoine genes in *Halomonas H. smyrnensis* AAD6T, *H. elongate* and *Marinobacter* sp. CP1. Note: Sugar phosphate permease (mfs^{cp}), Aspartokinase (*ask*), sodium-solute symporter family protein (SSS), ABC transporter (ABC), drug resistance transporter EMrB/QacA subfamily (*emrB*), choline transporter (*BetH*), choline dehydrogenase (*BetA*), glycine betaine aldehyde dehydrogenase (*BetB*), transcriptional regulator (*BetI*), L-2,4 diaminobutyric acid acetyltransferase (*ectA*), Diaminobutyratepyruvate aminotransferase (*ectB*), L-ectoine synthase (*ectC*) and ectoine hydrolase (*ectD*) [9].

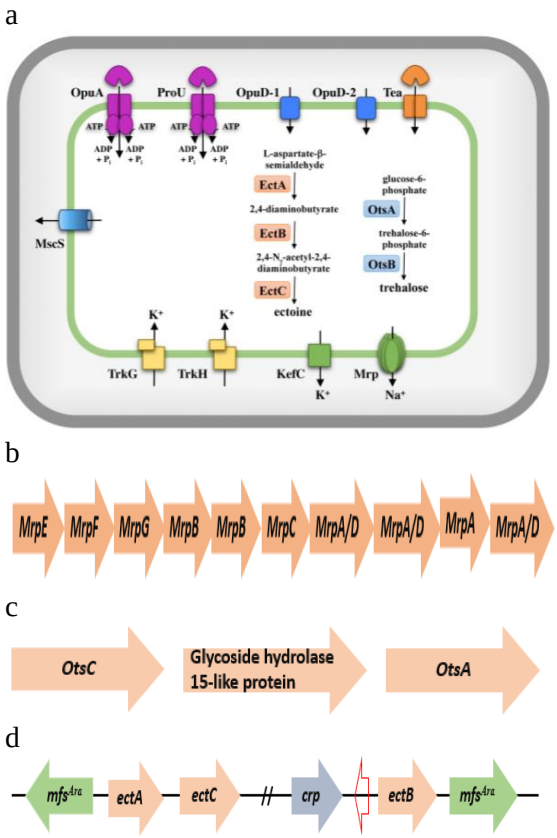

Figure 7. (a) Compatible solute uptake and biosynthesis (b) Mrp-system operon (c) Trehalose biosynthetic operon (d) Ectoine operon. Cation antiporter (*MrpE*), multiple resistance and pH regulation protein F (*MrpF*), monovalent cation/proton antiporter subunit MnhG/PhaG (*MrpG*), monovalent cation/H+ antiporter subunit B (*MrpB*), NADH-ubiquinonone oxidoreductase chain 4L (*MrpC*), monovalent cation/H+ antiporter subunit D and NADH dehydrogenase (quinone) (*MrpA/D*), L-2,4-diaminobytyric acid acetyltransferase (*ectA*), Diaminobutyratepyruvate aminotransferase (*ectB*), L-ectoine synthase (*ectC*), α, α-trehalose-phosphate synthase (*OtsA*), trehalose-6-phosphate phosphatase (*OtsB*) and transcriptional regulator (*crp*) [129].

taine aldehyde dehydrogenase (BetB) (Figure 6b). The genome analysis of *H. smyrnensis* AAD6T also revealed genes encoding ectoine biosynthesis. The genome possesses ectABCD arranged in an operon but ectD was located far from others. However, gene encoding a transcriptional regulator or aspartokinase was not found or located adjacent to ectABC genes, unlike other microbial genome such as *Marinobacter* sp. CP1 and *Halomonas elongate* genomes (Figure 6c).

Halophilic *Spiribacter salinus* M19-40 genome revealed both genes involved in two strategies of adaptation [129]. The genome has two copies of *Trk*-type K^+ uptake systems (*TrkG* and

TrkH). This type of K^+ importer has a membrane-embedded K^+ translocating subunit operating in conjunction with a peripheral membrane protein containing an RCK-domain (Regulation of K^+ channel), that forms a gating ring controlling the activity of the transporter. Also, gene related to K^+ efflux systems (*KefC*-type) and a multi-component *Mrp* Na^+ extrusion system which may be responsible for homeostasis of monovalent inorganic cation, energy metabolism and pH regulation (Figure 7a). However, the activity of *Mrp* system is very important for ensuring growth in high-salt environments [131]. In *Spiribacter salinus* M19-40, Mrp-system appears to be arranged as an operon comprising of 10 genes (Figure 7b). Simi larly, various kinds of compatible solute transporters in which five distinct systems were detected in the genome (Figure 7a). Two of these which predicted osmolyte importers are single components systems (*OpuD-1* and *OpuD-2*) and ABC transporters (*OpuA* and *ProU* systems) which are likely involved in the compatible uptake. *TeaABC*-type TRAP transporter was also detected which may function as an import system for ectoine and 5-hydroxyectoine (Figure 7a). Further inspection of the *Spiribacter salinus* M19-40 genome shows genes corresponding to trehalose biosynthesis (*OtsA* and *OtsB*), these genes transcribed as an osmotically inducible operon which are separated by a gene encodes for a family of the glycoside hydrolase 15 family (Figure 7c). In addition, the genome also encodes genes involved in ectoine biosynthesis which are typically organised in an operon, but they are not genetically organized into the canonical *ectABC* operon (Figure 7d). In this arrangement *ectA* and *ectB* genes are found close together and their function as part of the ectoine synthetic pathway seems to be direct while *ectB* gene was found to be clustered with predicted transcriptional regulator (*crp*) gene (Figure 7d).

Conclusion

High salinity halophiles had developed approaches to survive the osmotic stress of extreme saline conditions by intracellularly amassing inorganic ions (K^+) to counterbalance high salt concentration or synthesizing and accumulating the compatible solutes. These strategies for some halophiles are chloride ion dependent process. However, the advancement of new molecular techniques like metagenomics and next generation sequencing had transformed our understanding on microbial biology and discovery of novel genes involve. The microbial diversity of such hypersaline environments had been studied using culture-independent approaches that is metagenomic techniques. Little had been done to determine arrangement of gene structure and regulation as well as discovery of novel genes involving in the molecular mechanism of their adaptation to such extreme environments using genomic/metagenomic approach. The prokaryotes dwelling hypersaline environments display an astonishing diversity and so much progress is required towards the understanding of their metabolism, gene arrangement and gene regulation.

For future perspective, is to establish operons involving growth in hypersaline conditions which will reveal their molecular mechanism of their adaptation to extreme salt conditions by means of metagenomic and genomic studies. And this complement with their physical properties observed in the laboratory as well.

Acknowledgement

This research was funded by the Fundamental Research Grant Scheme - Ministry of Education Malaysia R.J130000.7854.5F189 (FRGS/1/2019/STG05/UTM/01/1). Last but not least, HAO thanks Tertiary Education Trust Fund (TETFund) of Nigeria for studentship.

References

1. De Vitis V, Guidi B, Contente ML et al. (2015) Marine microorganisms as source of stereoselective esterases and ketoreductases: kinetic resolution of a prostaglandin intermediate. Marine Biotechnology 17 (2): 144-152.
2. Prakash J, Chandra P (2020) Halophilic Microbes from Plant Growing Under the Hypersaline Habitats and Their Application for Plant Growth and Mitigation of Salt Stress. In *Plant Microbiomes for Sustainable Agriculture* (pp. 317-349). Springer, Cham.
3. Mapelli F, Crotti E, Molinari F et al. (2016) Extreme marine environments (brines, seeps, and smokers). In *The Marine Microbiome* (pp. 251-282). Springer, Cham.
4. Antunes A, Olsson-Francis K, McGenity TJ (2020) Exploring Deep-Sea Brines as Potential Terrestrial Analogues of Oceans in the Icy Moons of the Outer Solar System. Current Issues in Molecular Biology 38: 123-162.

5. Gutierrez T, Morris G, Ellis D et al. (2020) Production and characterisation of a marine *Halomonas* surface-active exopolymer. Applied Microbiology and Biotechnology 104 (3): 1063-1076.
6. Oyewusi HA, Wahab RA, Huyop F (2020) Dehalogenase-producing halophiles and their potential role in bioremediation. Marine Pollution Bulletin 160: 111603.
7. Oyewusi HA, Abdul Wahab R, Edbeib MF et al. (2021). Functional profiling of bacterial communities in Lake Tuz using 16S rRNA gene sequences. Biotechnology & Biotechnological Equipment 35 (1): 1-10.
8. Decho AW, Gutierrez T (2017) Microbial extracellular polymeric substances (EPSs) in ocean systems. Frontiers in Microbiology 8: 922.
9. Diken E, Ozer T, Arikan M et al. (2015) Genomic analysis reveals the biotechnological and industrial potential of levan producing halophilic extremophile, *Halomonas smyrnensis* AAD6T. SpringerPlus 4 (1): 393.
10. Caruso C, Rizzo C, Mangano S et al. (2018). Production and biotechnological potential of extracellular polymeric substances from sponge-associated Antarctic bacteria. Applied and Environmental Microbiology, 84 (4): e01624-17.
11. Lozupone CA, Knight R (2007) Global patterns in bacterial diversity. Proceedings of the National Academy of Sciences 104 (27): 11436-11440.
12. Bebianno MJ, da Fonseca TG (2020) Fate and Effects of Cytostatic Pharmaceuticals in the Marine Environment. In *Fate and Effects of Anticancer Drugs in the Environment* (pp. 295-330).
13. Forster D, Behnke A, Stoeck T (2012) Meta-analyses of environmental sequence data identify anoxia and salinity as parameters shaping ciliate communities. Systematics and Biodiversity 10 (3): 277-288.
14. Bourillot R, Vennin E, Dupraz C et al. (2020) The Record of Environmental and Microbial Signatures in Ancient Microbialites: The Terminal Carbonate Complex from the Neogene Basins of Southeastern Spain. Minerals 10 (3): 276.
15. Litalien A, Zeeb B (2020) Curing the earth: A review of anthropogenic soil salinization and plant-based strategies for sustainable mitigation. Science of The Total Environment 698: 134235.
16. Bakka K, Challabathula D (2020) Amelioration of Salt Stress Tolerance in Plants by Plant Growth-Promoting Rhizobacteria: Insights from "Omics" Approaches. In *Plant Microbe Symbiosis* (pp. 303-330).
17. McGenity TJ, Oren A (2012) Hypersaline environments. Life at extremes: environments, organisms and strategies for survival: 402-437.
18. Weinisch L, Kühner S, Roth R et al. (2018) Identification of osmoadaptive strategies in the halophile, heterotrophic ciliate *Schmidingerothrix salinarum*. PLoS Biology 16 (1): e2003892.
19. Niero M, Righetto I, Beneventi E .et al (2020) Unique Features of a New Baeyer–Villiger Monooxygenase from a Halophilic Archaeon. Catalysts 10 (1): 128.
20. Sharma AK, Kikani BA, Singh SP (2020) Biochemical, thermodynamic and structural characteristics of a biotechnologically compatible alkaline protease from a haloalkaliphilic, *Nocardiopsis dassonvillei* OK-18. International Journal of Biological Macromolecules.
21. Silva C, Martins M, Jing S et al. (2018) Practical insights on enzyme stabilization. Critical Reviews in Biotechnology 38 (3): 335-350.
22. Jin M, Gai Y, Guo X et al. (2019) Properties and applications of extremozymes from deep-sea extremophilic microorganisms: A mini review. Marine Drugs 17 (12): 656.
23. Schmid AK, Allers T, DiRuggiero J (2020) SnapShot: Microbial Extremophiles. Cell 180 (4): 818-818.
24. Chen S, Xu Y, Helfant L (2020) Geographical Isolation, Buried Depth, and Physicochemical Traits Drive the Variation of Species Diversity and Prokaryotic Community in Three Typical Hypersaline Environments. Microorganisms 8 (1): 120.
25. Jebbar M, Hickman-Lewis K, Cavalazzi B et al. (2020) Microbial Diversity and Biosignatures: An Icy Moons Perspective. Space Science Reviews 216 (1): 10.
26. Varshney P, Mikulic P, Vonshak A et al. (2015) Extremophilic micro-algae and their potential contribution in biotechnology. Bioresource Technology 184: 363-372.
27. Alavi S, Rafieyan S, Yavari-Bafghi M, Amoozegar MA (2020) Extremophiles: A Powerful Choice for Bioremediation of Toxic Oxyanions. In *Microbial Bioremediation & Biodegradation* (pp. 203-249). Springer, Singapore.
28. Oyewusi HA, Wahab RA, Kaya Y et al. (2020) Alternative bioremediation agents against haloacids, haloacetates and chlorpyrifos using novel halogen-degrading bacterial isolates from the Hypersaline Lake Tuz. Catalysts 10 (6): 651.
29. Oren A (2008) Microbial life at high salt concentrations: phylogenetic and metabolic diversity. Saline Systems 4 (1): 1-13.
30. Gunde-Cimerman N, Plemenitaš A, Oren A (2018) Strategies of adaptation of microorganisms of the three domains of life to high salt concentrations. FEMS Microbiology Reviews 42 (3): 353-375.
31. Weinisch L, Kirchner I, Grimm M et al. (2019) Glycine betaine and ectoine are the major compatible solutes used by four different halophilic heterotrophic ciliates. Microbial Ecology 77(2): 317-331.
32. Zeaiter Z, Marasco R, Booth JM et al. (2019) Phenomics and genomics reveal adaptation of Virgibacillus dokdonensis strain 21D to its origin of isolation, the seawater-brine interface of the Mediterranean Sea deep hypersaline anoxic basin Discovery. Frontiers in Microbiology 10: 1304.
33. Huan R, Huang J, Liu D et al. (2019) Genome sequencing of Mesonia algae K4-1 reveals its adaptation to the Arctic ocean. Frontiers in Microbiology 10: 2812.
34. Martínez-Espinosa RM (2020) Heterologous and Homologous Expression of Proteins from Haloarchaea: Denitrification as Case of Study. International Journal of Molecular Sciences 21 (1): 82.
35. Yang HW, Song JY, Cho SM et al. (2020) Genomic Survey of Salt Acclimation-Related Genes in the Halophilic *Cyanobacterium Euhalothece* sp. Z-M001. Scientific Reports 10.
36. Oren A (2006) Life at high salt concentrations. The Prokaryotes, 3, 263-282.
37. Mevarech M, Frolow F, Gloss LM (2000) Halophilic enzymes: proteins with a grain of salt. Biophysical Chemistry 86 (2-3): 155-164.
38. DasSarma S, DasSarma P (2015) Halophiles and their enzymes: negativity put to good use. Current Opinion

in Microbiology 25: 120-126.
39. Bayley ST, Morton RA, Lanyi JK (1978) Recent developments in the molecular biology of extremely halophilic bacteria. CRC Critical Reviews in Microbiology 6 (2): 151-206.
40. Baliga NS, Bonneau R., Facciotti MT et al. (2004) Genome sequence of *Haloarcula marismortui*: a halophilic archaeon from the Dead Sea. Genome Research 14(11): 2221-2234.
41. DasSarma SL, Capes MD, DasSarma P, DasSarma S (2010) HaloWeb: the haloarchaeal genomes database. Saline Systems, 6(1): 12.
42. Bolhuis A, Kwan D, Thomas JR (2008) Halophilic adaptations of proteins. Protein Adaptation in Extremophiles: 71-104.
43. Mavromatis K, Ivanova N, Anderson I et al. (2009). Genome analysis of the anaerobic thermohalophilic bacterium *Halothermothrix orenii*. PLoS One 4(1).
44. Galinski EA, Trüper HG (1994) Microbial behaviour in salt-stressed ecosystems. FEMS Microbiology Reviews 15(2-3): 95-108.
45. Saum R, Mingote A, Santos H, Müller V (2009) A novel limb in the osmoregulatory network of Methanosarcina mazei Gö1: Nε-acetyl-β-lysine can be substituted by glutamate and alanine. Environmental Microbiology 11(5): 1056-1065.
46. Ventosa A, Nieto JJ, Oren A (1998) Biology of moderately halophilic aerobic bacteria. Microbiology and Molecular Biology Reviews 62(2): 504-544
47. Lanyi JK (1974) Salt-dependent properties of proteins from extremely halophilic bacteria. Bacteriological Reviews 38(3): 272.
48. Oren A (1999) Bioenergetic aspects of halophilism. Microbiological and Molecular Biology Reviews 63(2): 334-348.
49. Saum SH, Müller V (2008) Regulation of osmoadaptation in the moderate halophile *Halobacillus halophilus*: chloride, glutamate and switching osmolyte strategies. Saline Systems 4(4): 2014.
50. Kixmüller D, Greie JC (2012) An ATP-driven potassium pump promotes long-term survival of *Halobacterium salinarum* within salt crystals. Environmental Microbiology Reports, 4(2): 234-241.
51. Oren A, Larimer F, Richardson P et al. (2005) How to be moderately halophilic with broad salt tolerance: clues from the genome of *Chromohalobacter salexigens*. Extremophiles 9(4): 275-279.
52. Oren A, Heldal M, Norland S, Galinski EA (2002) Intracellular ion and organic solute concentrations of the extremely halophilic bacterium *Salinibacter ruber*. Extremophiles 6(6): 491-498.
53. Mongodin EF, Nelson KE, Daugherty S et al. (2005) The genome of Salinibacter ruber: convergence and gene exchange among hyperhalophilic bacteria and archaea. Proceedings of the National Academy of Sciences 102(50): 18147-18152.
54. Wood JM, Bremer E, Csonka LN et al. (2001). Osmosensing and osmoregulatory compatible solute accumulation by bacteria. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 130(3): 437-460.
55. Saum SH, Müller V (2008) Growth phase-dependent switch in osmolyte strategy in a moderate halophile: ectoine is a minor osmolyte but major stationary phase solute in *Halobacillus halophilus*. Environmental Microbiology 10**:** 716–726.
56. Vargas C, Jebbar M, Carrasco R et al. (2006) Ectoines as compatible solutes and carbon and energy sources for the halophilic bacterium *Chromohalobacter salexigens*. Journal of Applied Microbiology 100(1): 98-107.
57. Petrovicˇ U, Gunde-Cimerman N, Plemenitasˇ A (2002) Cellular responses to environmental salinity in the halophilic black yeast Hortaea werneckii. Molecular Microbiology 45(3): 665-672.
58. Roeßler M, Müller V (2001) Chloride dependence of glycine betaine transport in Halobacillus halophilus. FEBS letters 489(2-3): 125-128.
59. Saum SH, Sydow JF, Palm P et al. (2006) Biochemical and molecular characterization of the biosynthesis of glutamine and glutamate, two major compatible solutes in the moderately halophilic bacterium *Halobacillus halophilus*. Journal of Bacteriology 188(19): 6808-6815.
60. Saum SH, Müller V (2007) Salinity-dependent switching of osmolyte strategies in a moderately halophilic bacterium: glutamate induces proline biosynthesis in *Halobacillus halophilus*. Journal of Bacteriology 189: 6968–6975.
61. Inbar L, Lapidot A (1988) The structure and biosynthesis of new tetrahydropyrimidine derivatives in actinomycin D producer *Streptomyces parvulus*. Use of 13C- and 15N-labeled L-glutamate and 13C and 15N NMR spectroscopy. Journal of Biological Chemistry 263: 16014–16022.
62. Bursy J, Pierik, AJ, Pica N, Bremer E (2007) Osmotically induced synthesis of the compatible solute hydroxyectoine is mediated by an evolutionarily conserved ectoine hydroxylase. Journal of Biological Chemistry 282: 31147–31155
63. Yancey PH (2005) Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. Journal of Experimental Biology, 208(15): 2819-2830.
64. Styrvold OB, Falkenberg P, Landfald B et al. (1986) Selection, mapping, and characterization of osmoregulatory mutants of Escherichia coli blocked in the choline-glycine betaine pathway. Journal of Bacteriology 165(3): 856-863.
65. Hoffmann T, Bremer E (2017) Guardians in a stressful world: the Opu family of compatible solute transporters from Bacillus subtilis. Biological Chemistry 398(2): 193-214.
66. Kappes R M, Kempf B, Kneip S et al. (1999) Two evolutionarily closely related ABC transporters mediate the uptake of choline for synthesis of the osmoprotectant glycine betaine in Bacillus subtilis. Molecular Microbiology, 32(1): 203-216.
67. Nyyssölä A, Kerovuo J, Kaukinen P et al. (2000) Extreme halophiles synthesize betaine from glycine by methylation. Journal of Biological Chemistry 275(29): 22196-22201.
68. Waditee R, Bhuiyan MNH, Rai V et al. (2005) Genes for direct methylation of glycine provide high levels of

glycinebetaine and abiotic-stress tolerance in Synechococcus and Arabidopsis. Proceedings of the National Academy of Sciences 102(5): 1318-1323.
69. Kimura Y, Kawasaki S, Yoshimoto H, Takegawa K (2010) Glycine betaine biosynthesized from glycine provides an osmolyte for cell growth and spore germination during osmotic stress in *Myxococcus xanthus*. Journal of Bacteriology 192(5): 1467-1470.
70. Strom AR, Kaasen I (1993) Trehalose metabolism in Escherichia coli: stress protection and stress regulation of gene expression. Molecular Microbiology 8(2): 205-210.
71. Kolman MA, Nishi CN, Perez-Cenci M, Salerno GL (2015) Sucrose in cyanobacteria: from a salt-response molecule to play a key role in nitrogen fixation. Life 5(1): 102-126.
72. Kosar F, Akram NA, Sadiq M et al. (2019) Trehalose: a key organic osmolyte effectively involved in plant abiotic stress tolerance. Journal of Plant Growth Regulation 38(2): 606-618.
73. Hasan H, Gul A, Amir R et al. (2020) Role of osmoprotectants and drought tolerance in wheat. In *Climate Change and Food Security with Emphasis on Wheat* (pp. 207-216). Academic Press.
74. Purvis JE, Yomano LP, Ingram LO (2005) Enhanced trehalose production improves growth of Escherichia coli under osmotic stress. Applied and Environmental Microbiology, 71(7): 3761-3769.
75. Cheng HJ, Sun YH, Chang HW et al. (2020) Compatible solutes adaptive alterations in Arthrobacter simplex during exposure to ethanol, and the effect of trehalose on the stress resistance and biotransformation performance. Bioprocess and Biosystems Engineering: 1-14.
76. Mitchell WJ (2015) The phosphotransferase system in solventogenic clostridia. Journal of Molecular Microbiology and Biotechnology, 25(2-3): 129-142.
77. Mitchell WJ (2016) Sugar uptake by the solventogenic clostridia. World Journal of Microbiology and Biotechnology 32(2): 32.
78. Ohtani K, Shimizu T (2015) Regulation of toxin gene expression in Clostridium perfringens. Research in Microbiology 166(4): 280-289.
79. Baker JL, Lindsay EL, Faustoferri RC et al. (2018) Characterization of the trehalose utilization operon in Streptococcus mutans reveals that the TreR transcriptional regulator is involved in stress response pathways and toxin production. Journal of Bacteriology 200(12): e00057-18.
80. Park M, Mitchell WJ, Rafii F (2016) Effect of trehalose and trehalose transport on the tolerance of Clostridium perfringens to environmental stress in a wild type strain and its fluoroquinolone-resistant mutant. International Journal of Microbiology: 2016.
81. Wang HL, Sun L (2017) Comparative metagenomics reveals insights into the deep-sea adaptation mechanism of the microorganisms in Iheya hydrothermal fields. World Journal of Microbiology and Biotechnology 33(5): 86.
82. Bürklen L, Schöck F, Dahl MK (1998) Molecular analysis of the interaction between the Bacillus subtilis trehalose repressor TreR and the tre operator. Molecular and General Genetics MGG 260(1): 48-55.
83. Avci MK, Yamaner C, Ayvaz M, Yazgan-Karatas A (2014) In silico characterization and comparative analysis of Bacillus subtilis GntR type LutR transcription factor. EurAsian Journal of BioSciences (8).
84. Avonce N, Mendoza-Vargas A, Morett E, Iturriaga G (2006) Insights on the evolution of trehalose biosynthesis. BMC Evolutionary Biology 6(1): 109.
85. Müller V, Oren A (2003) Metabolism of chloride in halophilic prokaryotes. Extremophiles 7(4): 261-266.
86. Roeβler M, Müller V (2002) Chloride, a new environmental signal molecule involved in gene regulation in a moderately halophilic bacterium, Halobacillus halophilus. Journal of Bacteriology 184(22): 6207-6215
87. Antón J, Oren A, Benlloch S et al. (2002) Salinibacter ruber gen. nov., sp. nov., a novel, extremely halophilic member of the Bacteria from saltern crystallizer ponds. International Journal of Systematic and Evolutionary Microbiology 52(2): 485-491
88. Burkhardt J, Sewald X, Bauer B et al. (2009) Synthesis of glycine betaine from choline in the moderate halophile *Halobacillus halophilus*: co-regulation of two divergent, polycistronic operons. Environmental Microbiology Reports 1(1): 38-43.
89. Saum SH, Pfeiffer F, Palm P et al. (2013) Chloride and organic osmolytes: a hybrid strategy to cope with elevated salinities by the moderately halophilic, chloride-dependent bacterium *Halobacillus halophilus*. Environmental Microbiology 15(5), 1619-1633.
90. Saum SH, Roeßler M, Koller C et al. (2007) Glutamate restores growth but not motility in the absence of chloride in the moderate halophile *Halobacillus halophilus*. Extremophiles 11 (5): 711-717.
91. Schloss PD, Handelsman J (2005) Metagenomics for studying unculturable microorganisms: cutting the Gordian knot. Genome Biology 6(8): 229.
92. Amann, RI, Binder BJ, Olson RJ et al. (1990) Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Applied and Environmental Microbiology 56 (6): 1919-1925.
93. Fujita MJ, Kimura N, Sakai A et al. (2011). Cloning and heterologous expression of the vibrioferrin biosynthetic gene cluster from a marine metagenomic library. Bioscience, Biotechnology, and Biochemistry 75 (12): 2283-2287.
94. Fujita MJ, Kimura N, Yokose H, Otsuka M (2012) Heterologous production of bisucaberin using a biosynthetic gene cluster cloned from a deep sea metagenome. Molecular BioSystems 8 (2): 482-485.
95. Gupta SK, Rai AK, Sarim KM et al. (2019) Metaproteomic data of maize rhizosphere for deciphering functional diversity. Data in Brief 27: 104574.
96. Lou J, Liu M, Gu J et al. (2019) Metagenomic sequencing reveals microbial gene catalogue of phosphinothricin-utilized soils in South China. Gene 711: 143942.
97. Deng Y, Ruan Y, Ma B et al. (2019) Multi-omics analysis reveals niche and fitness differences in typical denitrification microbial aggregations. Environment International 132: 105085.
98. Li W, Zhuang JL, Zhou YY et al. (2020) Metagenomics reveals microbial community differences lead to differential nitrate production in anammox reactors with differing nitrogen loading rates. Water Research 169: 115279.

99. Robbins RJ, Krishtalka L, Wooley JC (2016) Advances in biodiversity: metagenomics and the unveiling of biological dark matter. Standards in Genomic Sciences, 11 (1): 69.
100. Vera-Gargallo B, Ventosa A (2018) Metagenomic Insights into the Phylogenetic and Metabolic Diversity of the Prokaryotic Community Dwelling in Hypersaline Soils from the Odiel Saltmarshes (SW Spain). Genes 9 (3): 152.
101. Sarsaiya S, Jain A, Jia Q et al. (2020) Molecular Identification of Endophytic Fungi and Their Pathogenicity Evaluation Against *Dendrobium nobile* and *Dendrobium officinale*. International Journal of Molecular Sciences 21 (1): 316.
102. Duru IC, Laine P, Andreevskaya M et al. (2018). Metagenomic and metatranscriptomic analysis of the microbial community in Swiss-type Maasdam cheese during ripening. International Journal of Food Microbiology 281: 10-22.
103. Falk N, Reid T, Skoyles A et al. (2019) Microbial metatranscriptomic investigations across contaminant gradients of the Detroit River. Science of the Total Environment 690: 121-131.
104. Ma L, Wang H, Wu J et al. (2019). Metatranscriptomics reveals microbial adaptation and resistance to extreme environment coupling with bioleaching performance. Bioresource Technology 280: 9-17.
105. Becker EA, Seitzer PM, Tritt A et al. (2014). Phylogenetically driven sequencing of extremely halophilic archaea reveals strategies for static and dynamic osmoresponse. PLoS Genetics 10 (11).
106. Pérez-Llano Y, Rodríguez-Pupo EC, Druzhinina IS et al. (2020) Stress Reshapes the Physiological Response of Halophile Fungi to Salinity. Cells 9 (3): 525.
107. Oberbeckmann S, Labrenz M (2020) Marine microbial assemblages on microplastics: Diversity, adaptation, and role in degradation. Annual Reviews.
108. Li X, Zhu YG, Shaban B et al. (2015) Assessing the genetic diversity of Cu resistance in mine tailings through high-throughput recovery of full-length *copA* genes. Scientific Reports *5*: 13258.
109. Fernández AB, Ghai R, Martin-Cuadrado AB et al. (2014) Prokaryotic taxonomic and metabolic diversity of an intermediate salinity hypersaline habitat assessed by metagenomics. FEMS Microbiology Ecology 88 (3): 623-635.
110. Ghai R, Pašić L, Fernández AB, Martin-Cuadrado AB et al. (2011) New abundant microbial groups in aquatic hypersaline environments. *Scientific Reports*, *1*, 135.
111. Fernández AB, Vera-Gargallo B, Sánchez-Porro C et al. (2014). Comparison of prokaryotic community structure from Mediterranean and Atlantic saltern concentrator ponds by a metagenomic approach. Frontiers in Microbiology 5: 196.
112. Fernández AB, León MJ, Vera B et al. (2014). Metagenomic sequence of prokaryotic microbiota from an intermediate-salinity pond of a saltern in Isla Cristina, Spain. Genome Announcements 2 (1): e00045-14. doi: 10.1128/genomeA.00045-14.
113. Podell S, Ugalde JA, Narasingarao P et al. (2013). Assembly-driven community genomics of a hypersaline microbial ecosystem. PLoS One 8 (4): e61692.
114. Maturrano L, Santos F, Rosselló-Mora R, Antón J (2006) Microbial diversity in Maras salterns, a hypersaline environment in the Peruvian Andes. Applied and Environmental Microbiology 72 (6): 3887-3895
115. López-López A, Yarza P, Richter M et al. (2010) Extremely halophilic microbial communities in anaerobic sediments from a solar saltern. Environmental Microbiology Reports 2 (2): 258-271.
116. Dillon JG, Carlin M, Gutierrez A et al. (2013) Patterns of microbial diversity along a salinity gradient in the Guerrero Negro solar saltern, Baja CA Sur, Mexico. Frontiers in Microbiology 4: 399.
117. Ruvindy R, White III RA, Neilan BA, Burns BP (2016) Unravelling core microbial metabolisms in the hypersaline microbial mats of Shark Bay using high-throughput metagenomics. The ISME Journal 10 (1): 183.
118. Yau S, Lauro FM, Williams TJ et al. (2013) Metagenomic insights into strategies of carbon conservation and unusual sulfur biogeochemistry in a hypersaline Antarctic lake. The ISME Journal 7 (10): 1944.
119. Fierer N, Leff JW, Adams BJ et al. (2012). Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. Proceedings of the National Academy of Sciences 109 (52): 21390-21395.
120. La Cono V, Smedile F, Bortoluzzi G, Arcadi E et al. (2011). Unveiling microbial life in new deep-sea hypersaline Lake Thetis. Part I: Prokaryotes and environmental settings. Environmental Microbiology 13 (8): 2250-2268.
121. Pachiadaki MG, Yakimov MM, LaCono V et al. (2014). Unveiling microbial activities along the halocline of Thetis, a deep-sea hypersaline anoxic basin. The ISME Journal 8 (12): 2478-2489.
122. Vavourakis CD, Ghai R, Rodriguez-Valera F et al. (2016). Metagenomic insights into the uncultured diversity and physiology of microbes in four hypersaline soda lake brines. Frontiers in Microbiology 7: 211.
123. Jacob JH, Hussein EI, Shakhatreh MAK, Cornelison CT (2017) Microbial community analysis of the hypersaline water of the Dead Sea using high-throughput amplicon sequencing. Microbiology Open 6 (5): e00500.
124. Tringe SG, Von Mering C, Kobayashi A et al. (2005). Comparative metagenomics of microbial communities. Science 308 (5721): 554-557.
125. Nayfach S, Pollard KS (2016) Toward accurate and quantitative comparative metagenomics. Cell 166 (5): 1103-1116.
126. Oyewusi HA, Wahab RA, Huyop F (2021) Whole genome strategies and bioremediation insight into dehalogenase-producing bacteria. Molecular Biology Reports: 1-15.
127. López-Pérez M, Ghai R, Leon MJ et al. (2013). Genomes of “Spiribacter”, a streamlined, successful halophilic bacterium. BMC Genomics 14 (1): 787.
128. León MJ, Ghai R, Fernandez AB et al. (2013) Draft genome of Spiribacter salinus M19-40, an abundant gammaproteobacterium in aquatic hypersaline environments. Genome Announcement 1 (1): e00179-12.
129. León MJ, Hoffmann T, Sánchez-Porro C et al. (2018). Compatible solute synthesis and import by the moderate halophile *Spiribacter salinus*: Physiology and genomics. Frontiers in Microbiology 9: 108.

130. John J, Siva V, Richa K et al. (2019). Life in High Salt Concentrations with Changing Environmental Conditions: Insights from Genomic and Phenotypic Analysis of *Salinivibrio* sp. Microorganisms 7 (11): 577.

Górecki K, Hägerhäll C, Drakenberg T (2014) The Na+ transport in gram-positive bacteria defect in the Mrp antiporter complex measured with 23Na nuclear magnetic resonance. Analytical Biochemistry 445: 80-86.