

Maulin P. Shah
Satarupa Dey *Editors*

Trends in Biotechnology of Polyextremophiles

 Springer

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ISBN 978-3-031-55031-7 ISBN 978-3-031-55032-4 (eBook)
<https://doi.org/10.1007/978-3-031-55032-4>

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*We dedicate this book to our parents, family,
and teachers for giving us the guidance and
motivation to complete this book*

Preface

Polyextremophiles are versatile organisms which can tolerate multiple stress in the surrounding environment. The advent of metagenomics and transcriptomics has provided us with better understanding of the diversity of these microorganisms in nature and provided us a detailed insight into their different metabolic activity. A continuous change in environment, rise in temperature, and melting of polar caps are jeopardizing the existence of these extremophilic microorganisms and leading to loss of their diversity. This loss of diversity may lead to the loss of wide range of commercially important bioactive compounds and metabolites obtained from them. This aspect of impact of rapid environmental changes on their diversity needs more attention. This current book deals with the latest development in the study of the diversity of the extremophiles and impact of environmental changes on them.

This book comprises a total of 19 chapters which address in detail the aspects of polyextremophiles and their mechanism to tolerate multiple stress in environment. Few other aspects such as bio-prospecting and bio-product development, bioremediation using extremophilic microbes are also discussed in brief. The various aspects of polyextremophiles can serve as a valuable source of information for scientists and students in a number of disciplines, including geology, chemistry, microbiology, pharmaceutical industry, environmental, and agricultural sectors. Moreover, their impact and importance in newly developing fields like astrobiology is also discussed.

We are grateful and acknowledge our deepest gratitude to all the contributing authors and the reviewers who supported immensely in shaping this book. We wholeheartedly express our thanks to all the authors for their efforts in writing wonderful chapters for this book. We also express our thanks to all the reviewers who have given their vital input to improve the chapters. We sincerely hope that people will share our pride in this work. We would also like to thank Springer Nature and all the team members for giving us this opportunity to edit this book. We would like to express our gratitude to our mentors, teachers, and students who have always guided and motivated us. We are thankful to them for providing us the assurance

and strength to complete our assignment. We also want to thank our coworkers, family, and friends for their encouragement and support during the preparation of this book.

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Acknowledgment

We are grateful and acknowledge our in-depth gratitude to all the contributors and the reviewers who trusted us and supported immensely in shaping this book. This book would not have been a success without vital contributions from the authors. We wholeheartedly express our thanks to all the authors for their efforts in writing wonderful chapters for this book. I also express my thanks to all the reviewers who have given their vital input to improve the chapters. We hope that they will be as proud of this book as we are. We would also like to thank Springer Nature and all the team members for giving us this opportunity to edit this book. We would like to express our gratitude to our mentors, teachers, and students who have always guided and motivated us. We convey our thanks to them for giving us the confidence and strength to accomplish this task. Also, we would like to thank our colleagues, family, and friends who provided encouragement and support during the work on this book.

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Overview of the Genes Associated with Polyextremophiles



Purbita Sikdar, Mahima Saha, Rounak Saha, and Debanjan Ghosh

Abstract Polyextremophiles are known to be a class of organisms that have the extraordinary ability to survive in multiple extreme environmental conditions. Their genetic makeup is what has set them apart from other Archaea and extremophiles in general. Among extremophiles, acido, thermo, halo, and similar other groups of organisms have already been discovered. These organisms have a unique composition of genes that help them survive in such extremities. Organisms such as thermoacidophiles, besides surviving in high temperatures, can also thrive in extremely low pH. Thus, these adaptations give us a glimpse of highly stable genetic content as well as metabolic flexibility in polyextremophiles. The maintenance of homeostasis in polyextremophiles such as thermoalkaliphiles and haloacidophiles is a great mystery for microbiologists. Membrane proteins, chaperons, and DNA repair proteins are greatly altered from that of their mesophilic counterparts. Thus, in this following chapter, we will try to analyze the genetic parameters that help these organisms create and exist in their own niche.

Mahima Saha and Rounak Saha contributed equally with all other contributors.

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M. P. Shah, S. Dey (eds.), *Trends in Biotechnology of Polyextremophiles*, https://doi.org/10.1007/978-3-031-55032-4_1

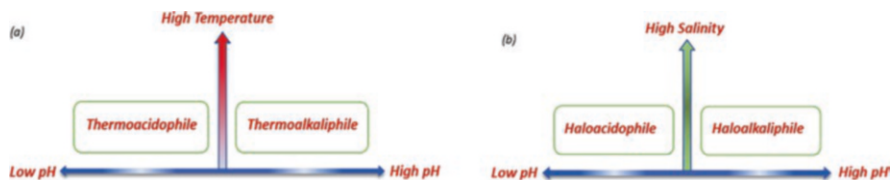


Fig. 1 Polyextremophiles at the interface of (a) temperature and pH and (b) salinity and pH. Referenced from (Seckbach et al. 2013)

1 Polyextremophiles: Environmental Extremities in Conjunction

Polyextremophiles are a class of microorganisms that have the unique capability of surviving in extremely hostile environments. Unlike extremophiles viz., acidophiles, alkaliphiles, thermophiles, etc., polyextremophilic organisms have the stunning ability to withstand more than one extreme condition, hence the name polyextremophile. The terrestrial environments they inhabit are a combination of multiple such hostile conditions, viz., temperature, pressure, pH extremities, and so on. These parameters have a direct influence on the basic needs of life, such as nutrient availability, thereby differentially affecting the microbial diversity of that niche. This establishes that life acclimatizes to an extensive range of parameters, independently and combined (Merino et al. 2019).

The major environmental extremes, temperature, pH, and salinity have been studied extensively, which provides us with information on the survival mechanisms of polyextremophiles. Organisms at the interfaces of such extremes make way for the different groups of polyextremophiles (Fig. 1a, b). These organisms have a unique genetic makeup that helps them thrive in such hostile conditions.

2 Adaptations of the Polyextremophilic Life

Life at the interface of extreme environmental conditions involves unique coping mechanisms and adaptive abilities in the organisms (mostly archaea) that live in such conditions. These give rise to phenotypic and/or physiological characteristics that enable changes in membrane permeability and control stability of cell walls, intracellular proteins, and intracellular osmotic balance. These basic metabolic, biochemical, and physiological properties have an important role to play in the adaptations of polyextremophilic life (Mesbah and Wiegel 2012).

The parameters, or the individual extremes, that influence polyextremophilic life are temperature, pH, salinity, pressure, radiation, desiccation, and oxygen stress. Certain parameters, depending on the environment, can influence archaeal life, more than others. The commonly found factors, hence the extensively studied ones, are temperature, pH, and salinity, which will be the major focus areas of this chapter.

Genetic diversity of microbial life happens due to dynamism in ecological and genetic evolutionary processes. The various mechanisms involved are natural selection, gene mutations, gain-loss, and genetic drift. The genetic diversity hence likely regulates the adaptive features of that habitat, in this case, the polyextremophilic habitat (Banerjee et al. 2021). Recent studies in the past decade have reported the influence of horizontal gene transfer, dinucleotide abundance, purine loading, and relative synonymous codon usage bias on the sustenance of organisms in the extremities (Banerjee et al. 2021; Lynn et al. 2002).

3 Genetic Stability of Thermoacidophiles

3.1 Archaeal Thermoacidophiles

Thermoacidophiles are those microorganisms that survive in extremely acidic conditions and high temperatures simultaneously. Organisms that can grow in $\text{pH} \leq 4$ and temperature ranging from 60°C to 80°C , while also being able to withstand several other biologically deleterious conditions, fall under the group of thermoacidophiles (Lewis et al. 2021). The large majority of thermoacidophiles are Archaea belonging to the Thermoproteota and Euryarchaeota phyla, although, occasionally, bacterial and eukaryotic organisms have been discovered. This class of organisms is well-studied because they thrive surprisingly well in discrete microbial communities with low species diversity (Seckbach et al. 2013).

Thermoacidophilic archaea, from across the earth, have been seen to persist in unusually hot and acidic environments, viz., acidic vents, volcanic solfataras, acidothermal areas, hot springs, acid mine drainage sites, etc. Acidic environments generated due to geothermal activities have a high temperature ($\sim 65^\circ\text{C}$) along with extremely low pH and can therefore be ideal for thermoacidophiles to survive (Seckbach et al. 2013). Orders *Sulfolobales* and *Thermoplasmatales* from the phyla Thermoproteota and Euryarchaeota, respectively, mostly dominate these environments and, as a result, have also been extensively studied.

These unique organisms of the world were discovered more than 50 years ago, but only recent developments in sequencing techniques gave insight into their genomic data. These advancements made way for detailed discussions on the evolution, survival strategies, genetic tools, metabolic processes, and cell biology of the thermoacidophiles (Lewis et al. 2021). This chapter, henceforth, will be focusing on the genetic makeup of the archaeal genera *Sulfolobus*, *Acidianus*, and *Metallosphaera* belonging to the order Sulfolobales (Fig. 2) and the genus *Picrophilus* belonging to the order Thermoplasmatales (Fig. 3).

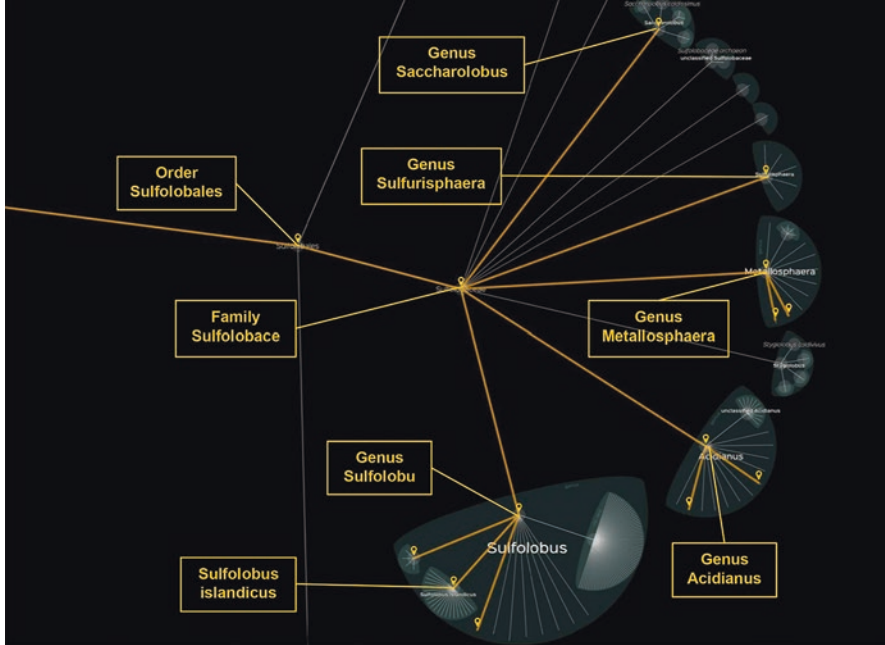


Fig. 2 Taxonomic classification of thermoacidophilic archaea under the Sulfolobales order (de Vienne 2016)

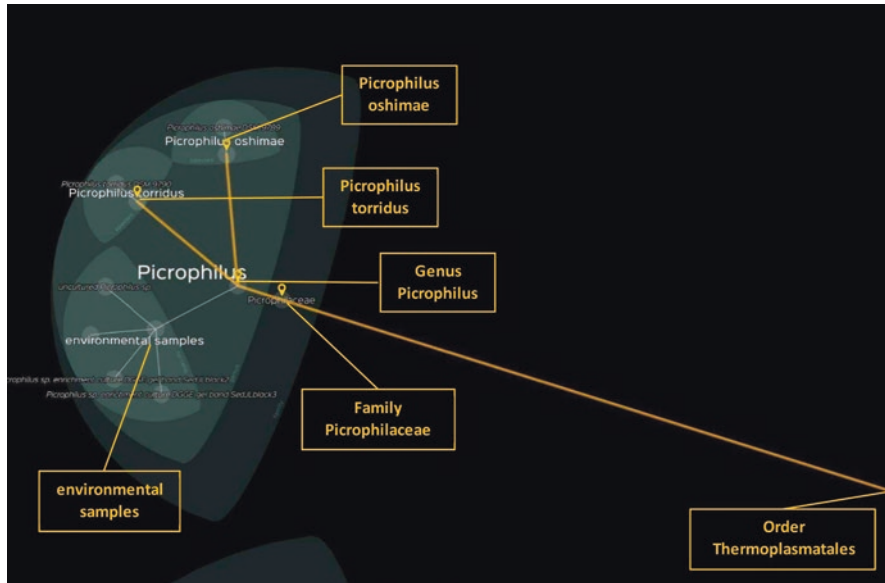


Fig. 3 Taxonomic classification of thermoacidophilic archaea under the Thermoplasmatales order (de Vienne 2016)

3.2 *Stress Response in Thermoacidophiles*

Thermoacidophilic archaea do not particularly consist of any stress response mechanism. They mostly cope with the extremities and essentially survive in them because of overlapped mechanisms to deal with thermal and acidic stress conditions.

Nucleotide and amino acid preferences: DNA stability of thermoacidophiles is not directly linked to higher G + C content. They instead have some preferred nucleotide base, codon, and/or amino acids to be thermally stable. They prefer the heat-stable purines A and G in their genome; the phenomenon is termed “nucleotide bias.” As a result, codons carrying more A/G than C/T are preferred for amino acid synthesis. For instance, for isoleucine, codon ATA would be preferred over ATT or ATC, and for arginine, codons AGA and AGG would be preferred over CGN (Singer and Hickey 2003). A preference for incorporating charged or hydrophobic amino acids while avoiding heat-labile, uncharged polar amino acids has been observed in thermophiles (Tekaia et al. 2002). Such usage of amino acids may be present in thermoacidophiles as well.

Heat Shock Proteins: Thermoacidophiles, like thermophiles, have a thermosome (molecular chaperone), a large HSP60-like protein complex, to cope with thermal stress. Along with it, small HSPs, HSP20, also serve as chaperones during thermal stress response. These small HSPs act by stabilizing proteins when exposed to high temperatures, thereby averting protein aggregation (Wang et al. 2010).

Reverse membrane potential: Both acidophiles and thermoacidophiles have mechanisms to maintain cytoplasmic neutrality while generating a proton force. This has led to the system of reverse membrane potential, where the intracellular membrane is positively charged and the extracellular membrane is negatively charged. Active transport of K^+ ions into the cell drives the reverse membrane potential, allowing acidophilic organisms to generate ATP despite considerable ΔpH across the membrane. However, this proton influx leads to acidification of the intracellular environment. This is prevented by proton pumps that export internalized proteins out of the cell (Lewis et al. 2021).

3.3 *The Sulfolobales*

The *Sulfolobales* order was the first archaeal lineage to be discovered. Thomas Brock in 1972 isolated a thermoacidophile from a sulfur hot spring at Yellowstone National Park. He then named the organism *Sulfolobus acidocaldarius* and designated the genus *Sulfolobus* (Lewis et al. 2021; Brock et al. 1972). After more than a decade, in 1986 and 1989, Karl Stetter established two more genera, namely, *Acidianus* and *Metallosphaera*, respectively. Both the organisms, *Acidianus infernus* and *Metallosphaera sedula*, were isolated from solfataras in Italy (Fig. 4).

The *Sulfolobus* genus consists of organisms that are obligate and facultative aerobes, chemoheterotrophs, and chemolithotrophs. The optimum temperature range

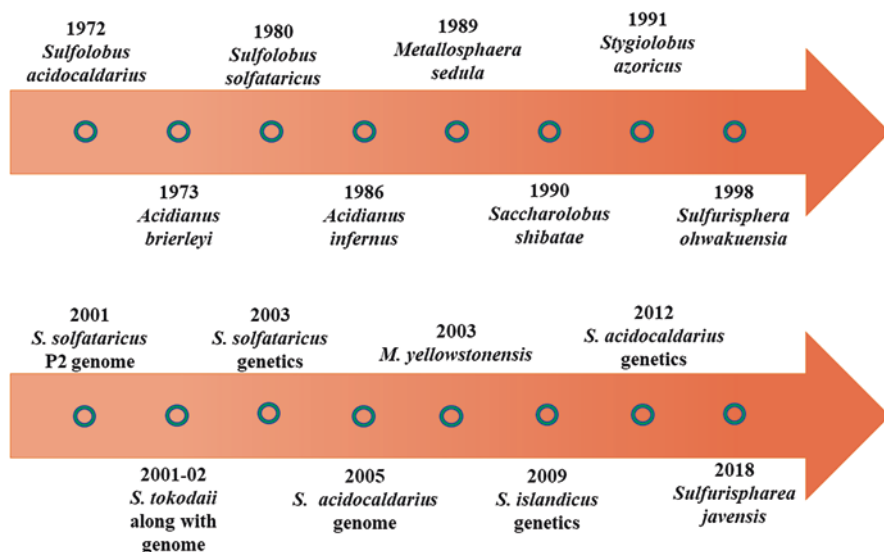


Fig. 4 Timeline of some of the major thermoacidophiles from the Sulfolobales order. Shows the year of isolation and the name of the isolate. Referenced from (Lewis et al. 2021)

of the archaea in this category is 65–88 °C, thus being classified as highly thermophilic, and the optimum pH ranges from 0.7 to 4.5, being moderately acidophilic (Lewis et al. 2021).

Over the course of time, seven genera of archaea have been discovered that, today, represent the Sulfolobales order; they are *Acidianus*, *Metallosphaera*, *Saccharolobus*, *Stygiolobus*, *Sulfodiicoccus*, *Sulfolobus*, *Sulfuracidifex*, and *Sulfurisphaera* (Counts et al. 2017; Lewis et al. 2021). Of these seven genera, the most studied are *Acidianus*, *Metallosphaera*, and *Sulfolobus*. The species representing these genera have been the focus of scientific research due to their similarities in the adaptation of extremities. The remaining genera are represented by a few species with varying characteristics.

3.4 Genus *Acidianus*

Organisms from this genus are anaerobic and sulfur-reducing. The genus contains the most acidophilic member *A. sulfidivorans* and the most thermophilic member *A. infernus*, of the *Sulfolobales* order known to date (Lewis et al. 2021).

Temperature range: 70–90 °C.

pH range: 0.8–2.5.

3.5 Genus *Metallosphaera*

This genus was named based on their ability to biooxidize iron. *M. prunae*, isolated from a uranium mine, has the ability to tolerate high levels of soluble uranium by employing a unique stress response mechanism (Lewis et al. 2021).

Temperature range: 65–75 °C.

pH range: 2.5–3.5.

3.6 Genus *Saccharolobus*

This genus considerably depends on sugar catabolism rather than on lithotrophic pathways as its sister genera. Some species, initially designated under *Sulfolobus* genus, proved to be evolutionary divergent and, hence, were assigned a separate genus, *Saccharolobus*. Members of this genus, like *Acidianus* genus, are also among the most thermophilic and least acidophilic organisms of the order.

Temperature optima: ≥ 80 °C.

pH optima: ≥ 3.0 .

Sulfolobus islandicus, an informally named species under the genus *Sulfolobus*, uses polysaccharides, pentoses, and hexoses, thereby being much more closely related to *Saccharolobus* (Grogan 1989).

3.7 Genus *Sulfolobus*

The most studied member of this genus is *Sulfolobus acidocaldarius*. It was discovered from a sulfur-rich solfataric field, hence the name. It utilizes sucrose, starch, dextrin, and amino acids and grows best at pH 3.0 and a temperature of 75 °C (Lewis et al. 2021). Only some of the members have a complete genome sequence and a tractable genetic system. They also have been extensively used as model systems for various studies to understand the *Thermoproteota* phyla.

3.8 CRISPR-Cas Mechanisms in Sulfolobales

CRISPR-Cas is the best-known defense mechanism strategy developed in archaea and bacteria. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) arrays are made of a series of ~30 base pair genomic repeat sequences, interspaced by sequences acquired from foreign genetic elements (van der Oost et al. 2014). Viral infections cause the integration of spacer sequences between repeats, which are snippets of the viral genome. The arrays are then processed by the Cas

(CRISPR-associated) proteins, and the spacers act as a reference/memory to interfere with subsequent viral infection (Wang and Doudna 2023).

Almost 85% of archaeal organisms consist of the CRISPR-Cas defense system. Organisms under the *Sulfolobales* order are particularly enriched with Class 1-type III system (Makarova et al. 2009).

3.9 Genetic Mechanisms and Adaptability

Genomic DNA organization machineries in archaea are related to their eukaryotic counterparts. Their gene regulation processes match with bacterial systems, while their chromosome organization has both bacterial and eukaryotic characteristics (Lewis et al. 2021).

Genome and genetic stability: The *Sulfolobales* genome comprises two distinct sub-megabase compartments that are morphologically reminiscent of eukaryotic chromosomes (Takemata et al. 2019). The transcriptionally active A compartment carries genes for core metabolic processes. The B compartment, on the other hand, is transcriptionally less active as compared to A, but is enriched in mobile genetic elements, viz., CRISPR-Cas clusters.

Insertion elements are highly mobile sequences responsible for high mutation frequency. *S. acidocaldarius*, *S. islandicus*, and *Sa. Solfataricus* are placed in the increasing order of genome instability. *S. acidocaldarius* exhibits only a small number of insertion elements, thus having lower mutation frequency and hence a more stable genome (Redder and Garrett 2006; Lewis et al. 2021).

Chromosome organization: The chromosome of thermoacidophilic archaea is relatively small, single, and circular in nature. It is packed into an organized chromatin structure by the action of various chromatin proteins (Lewis et al. 2021). Chromatin proteins present in the members of *Sulfolobales* are largely evolutionary divergent, with the absence of histone proteins, instead containing a variety of nucleoid-associated proteins (NAPs). NAPs comprise up to 5% of soluble cellular protein.

The *Sulfolobales* harbor two paralogs of the archaeal NAP Alba (Bell et al. 2002), a general nucleotide-binding protein coded by gene *alba*. Besides Alba, a highly conserved protein in the Thermoproteota phylum is Cren7, another NAP (Lewis et al. 2021).

Two such NAPs essential for the survival of *Sulfolobus* sp., Cren7 (Zhang et al. 2019) and Sso7d (Guagliardi et al. 2002), are described in detail below (Kuznetsov et al. 2022; Table 1):

Table 1 Cren7 and Sso7d, NAPs that are essential for the survival of *Sulfolobus* sp.

Protein name/ characteristics	Cren7	Sso7d
Gene name	creN7	–
Length	183 nts	195 nts
Features	<ul style="list-style-type: none"> • Versatile architectural protein responsible for chromatin condensation • Highly conserved nucleotide-binding protein in Thermoproteota 	<ul style="list-style-type: none"> • 7 kDa DNA binding protein • Contains high frequency of aromatic residues • Thermostable, highly expressed
Functions	<ul style="list-style-type: none"> • Preferentially binds AT-rich sequences of dsDNA • Protects duplex DNA from thermal denaturation • Constrains negative supercoil • Archaeal chromosomal packaging is Cren7 based 	<ul style="list-style-type: none"> • Promotes annealing of complementary DNA strands • Induces negative supercoiling, which is suggestive of protection of DNA from thermal denaturation

3.10 Central Dogma Machinery: Central Dogma Comprises DNA Replication, Transcription, and Translation

Replication: Archaeal lineages concerned in this chapter have striking similarity with eukaryotic replication machineries, i.e., containing multiple replication origins. Three replication origins (OriCs 1, 2, and 3), each being responsible for a single replication event, can be found in the DNA of *Sulfolobales*. Similar to the OriCs, replication initiator proteins in archaea, responsible for the assembly of replisome, resemble eukaryotic proteins (Lewis et al. 2021). Post replisome assembly, a replication-dedicated-DNA-dependent RNA polymerase sets off with primer synthesis for the leading or the lagging strand. The final eukaryote-like enzyme, DNA polymerase, responsible for DNA synthesis are of three different types, as seen in Thermoproteota (Lewis et al. 2021).

Transcription: The small archaeal genomes comprise an operonic transcription unit that is characterized by short intergenic regions, similar to a bacterial genetic organization. Extensive research on *Sulfolobales* members have led to certain findings regarding the transcription machinery and their evolutionary relationship with eukaryotic protein counterparts. The RNA polymerase enzymes of *Saccharolobus* sp. show structural similarity with eukaryotic RNA pol-II. Transcription factors of *Sulfolobales* members are also homologous to eukaryotic transcription factors (Lewis et al. 2021).

A few transcription regulators present in thermoacidophiles are tabulated below (Table 2):

Translation: The complex translation machinery of thermoacidophilic archaea also shows striking similarity with eukaryotes. The prevalent mechanism is based on the direct pairing of start codon with initiator anticodon in transcripts lacking the

Table 2 Transcription regulators of thermoacidophiles

Name	Species	Physiological role	Regulatory action
YtrA (Lemmens et al. 2019)	<i>Sulfolobus acidocaldarius</i>	Membrane protein expression	Repression
Lrp (Vassart et al. 2013)	<i>Sulfolobus acidocaldarius</i>	Metabolism and physiology regulation	Dual
LysM (Stoddard et al. 2014)	<i>Sulfolobus acidocaldarius</i>	Amino acid transport and metabolism	Activation
BldR2 (Fiorentino et al. 2011)	<i>Saccharolobus solfataricus</i>	Stress response to aromatic compounds	N.A.
HchR (Leyn et al. 2015)	<i>Metallosphaera yellowstonensis</i>	Autotrophic metabolism	N.A.
CopR/CopT (Villafane et al. 2011)	<i>Saccharolobus solfataricus</i>	Copper homeostasis	Repression

Shine-Dalgarno (SD) sequence. This is because polycistronic mRNAs in Sulfolobales are leaderless, lacking SD sequences.

Post-translational modifications are an efficient way to rapidly adjust protein functions according to changing internal and external signals. One of the most studied modifications is the reverse protein phosphorylation, of which the two-component system has been identified only in *Thermoplasmatales* and not in *Sulfolobales* (Lewis et al. 2021).

3.11 The *Thermoplasmatales*

Thermoplasmatales include the moderately thermophilic *Picrophilus* genus, containing two aerobic, heterotrophic organisms with remarkable acid tolerance. The members, first discovered from a dry solfatara in Japan, namely, *Picrophilus oshimae* and *Picrophilus torridus*, are the most acidophilic organisms known to date (Schleper et al. 1995; Angelov and Liebl 2006).

The temperature optima of the members of the *Picrophilus* genus is 60 °C, lower compared to other thermoacidophiles, with pH optima being 0. An unusual feature of these organisms is the striking difference between their intracellular pH and their optimum pH for growth being 4.6 and 0.7, respectively, which is in contrast to the neutral intracellular pH of other thermoacidophilic organisms (Angelov and Liebl 2006; Fütterer et al. 2004).

Of the two known species, only the complete genome of *P. torridus* has been sequenced (and published recently) (Fütterer et al. 2004). Along with the genome sequences of the five other known genera under thermoacidophiles, an extensive study can be performed for a better understanding of thermoacidophilic life and the use of horizontal gene transfer as an evolutionary mechanism.

3.12 Cellular Structure and Adaptation of *P. torridus*

The extremely hostile environments in which it lives directly impact the adaptability of the species. Apart from the adaptation mechanisms at metabolic, gene expression, and functional levels, *P. torridus* demonstrates the adaptability of cellular membrane and oxygen stress.

The cell membrane of *P. torridus* is highly acid stable, displays low proton permeability, and has been found to lose cell integrity at pH 7 through in vitro studies. It contains acid-resistant extracellular enzymes and genes for energy metabolism and electron transport chain. It also employs mechanisms to deal with oxygen stress to protect the cell from its aerobic requirements. The presence of such genes, pathways, and mechanisms in the species can be attributed to the horizontal gene transfer mechanism.

3.13 Genome of *P. torridus*

Shotgun sequencing was performed on the genomic DNA of *P. torridus* strain DSM 9790, the result of which is a completely sequenced genome that scientists now know. It is a 1,545,900-bp (1.5 Mb) large single circular chromosome (Fütterer et al. 2004), one of the smallest known genomes among non-parasitic free-living organisms (Angelov and Liebl 2006). Studies performed to date stand for the fact that a small genome size with a high coding density of *P. torridus* is influenced by the combination of two extreme living conditions, moderately high temperature, and exceptionally low pH (Table 3).

3.14 Replication Apparatus and Restriction System

While thermoacidophiles may tend to have similarities with both classic archaeal and eukaryotic cellular apparatuses, *P. torridus* has a classic archaeal-type replication apparatus. An ORF homolog recognizes the origin of replication, and DNA helicase, single-stranded binding proteins, topoisomerase, and DNA-polymerase complex proteins (X, B & D families) consecutively work for replicating the DNA (Fütterer et al. 2004).

Table 3 General features of the *P. torridus* genome (Fütterer et al. 2004)

General features	Number
Size, in base pairs	1,545,900
Coding region	91.7
G-C content (%)	36
Total no. of ORFs	1535

This species not only possesses the type II restriction/modification system; unlike other thermoacidophiles, it also has the type I system (Fütterer et al. 2004).

P. torridus genome lacks certain protein homologs, namely, nucleotide excision repair proteins, reverse gyrase, topoisomerase IV, and histone proteins, which questions their ability to maintain genome structure and integrity in hostile environments (Angelov and Liebl 2006). However, it does code for a large number of repair and recombination proteins that play a part in DNA repair and/or recombination processes (Fütterer et al. 2004).

3.15 Amino Acid Metabolism

The *P. torridus* genome contains biosynthetic pathways for all 20 amino acids and also contains certain genes and pathways for the degradation of amino acids. *P. oshimae*, on the other hand, has been reported to take up glutamate, serine, histidine, and proline for cellular needs (Fütterer et al. 2004). The cytoplasm of *P. oshimae* is also known to be a reservoir of acid-stable proteins, responsible for maintaining a low intracellular pH.

Both *P. torridus* and *S. solfataricus* bear a high number of ORFs that are homologous to each other.

3.16 Transport Mechanism

The genes of *P. torridus* code for transporters, with 12% dedicated to this function. These transporters include 17 primary and 93 secondary ones, maintaining a ratio of 1:5.6. Examples encompass channels for K^+ and Cl^- , as well as transporters for Fe^{3+} , Cu^{2+} , SO_4^{2-} , etc. It also possesses a K^+ -transporting ATPase, whose probable function is the uptake of potassium to create positive $\Delta\Psi$ (difference in water potential) inside the cell. This counteracts with the proton influx, thus coping with the high ΔpH (Fütterer et al. 2004).

An important finding is the high ratio of secondary to primary transporters in the species. It is a possible indication of the predominant use of a proton-driven secondary transport system demonstrating a decidedly relevant strategy for adaptation to extreme acidic conditions. Furthermore, the *Thermoplasmatales* possess the highest density of transport-encoding genes per Mb genome (Angelov and Liebl 2006). This adaptation strategy cannot be seen in the thermoacidophiles under the *Sulfolobales* order, hence proving to be a conserved trait among the former archaeal order, the *Thermoplasmatales* (Fütterer et al. 2004).

3.17 Energy Metabolism

A considerable amount of metabolic energy is possibly generated by the *P. torridus* species for it to maintain a low intracellular pH, while simultaneously confronting a huge proton gradient. Studies show the organism living like a scavenger, exploiting the decomposition products of other organisms in its hot and acidic environment (Angelov and Liebl 2006).

ED Pathway: The complete genome sequence has shed light on the energy metabolism processes of *P. torridus* to catabolize glucose that takes place via a non-phosphorylated variant of the commonly known Entner–Duodoroff (ED) Pathway (Angelov and Liebl 2006; Fütterer et al. 2004). For all the genes of this pathway, a gluconate dehydrogenase gene has been assigned to this genome, which is found to be orthologous to the KDG aldose gene in *S. solfataricus*, thus indicating a functional ED pathway in *P. torridus* (Fütterer et al. 2004).

EMP Pathway: The Embden–Meyerhoff–Parnas Pathway is supposedly used for gluconeogenesis with a non-classical fructose-1.6-bisphosphate aldolase. All the other genes required in this pathway are present as it is.

TCA Cycle: The Tricarboxylic Acid Cycle present in *P. torridus* is an operational pathway containing all the required genes. It also codes for propionyl-CoA synthase, which enables it to grow on propionate. Propionate functions as an uncoupler at low pH, thus being harmful to thermoacidophiles.

The presence of organic acid degradation pathways in these organisms therefore might play an important role in understanding the evolution of *P. torridus*.

3.18 Orthologs

Both the above-mentioned archaeal orders have been studied to a point where we can categorize them based on similarities as well as differences. One such categorization can be done based on orthologous genes found in the thermoacidophilic genera. The following part lists six such protein-coding genes found as orthologues in *Picrophilus*, *Sulfolobus*, *Metallosphaera*, *Saccharolobus*, and *Acidianus* (Kuznetsov et al. 2022).

1. Gene: *alba*

Length: 273 nts (nucleotides)

Codes for DNA-binding protein Alba. It is a highly conserved small nucleotide-binding protein among Archaea

Functional category: Transcription

Molecular function: double-stranded DNA binding

Eight single-copy genes are present in the *Thermoplasmatales*, with an average protein length of 89 aa, and 23 single-copy genes are present in the *Sulfolobales*, with an average protein length of 97 aa.

Genus/species: *P. torridus*, *Sulfolobus*, *Acidianus*, *Metallosphaera*

2. *Gene: queC*

Length: 720 nts (nucleotides)

Codes for 7-cyano-7-deazaguanine synthase QueC

Functional category: Translation, ribosomal structure, and biogenesis; cell wall/ membrane/envelope biogenesis; nucleotide transport and metabolism

Molecular function: Nucleotide binding; ATP binding; zinc ion binding; metal ion binding; ligase activity; C–N bond formation

Genus/species: *P. torridus*, *Sulfolobus acidocaldarius*, *M. sedula*

3. *Gene: ileS*

Length: 3075 nts (nucleotides).

Codes for isoleucine–tRNA ligase.

Biological processes: tRNA aminoacylation for protein translation; isoleucine–tRNA aminoacylation.

Molecular function: Isoleucine–tRNA ligase activity; aminoacyl tRNA editing activity; ATP binding; nucleotide binding.

Eight single-copy genes are present in the Thermoplasmatales, and 23 single-copy genes are present in the Sulfolobales.

Genus/species: *P. torridus*, *Sulfolobus*, *Acidianus*, *M. yellowstonensis*, *Saccharolobus*.

4. *Gene: cyoE*

Length: 870 nts (nucleotides).

Stands for heme O synthase.

Biological processes: Heme biosynthetic process; heme O biosynthetic process.

Molecular function: Protoheme IX farnesyltransferase; transferase activity.

Five single-copy genes are present in the Thermoplasmatales, and 24 single-copy genes are present in the Sulfolobales.

Genus/species: *P. torridus*, *S. acidocaldarius*, *S. islandicus*, *Sa. Solfataricus*, *M. yellowstonensis*.

5. *Cytochrome P450*

Length: 1149 nts (nucleotides)

Molecular function: Monooxygenase activity; oxidoreductase activity; metal ion binding; heme binding

Genus/species: *P. torridus*, *S. acidocaldarius*

6. *β -CASP ribonuclease aCPSF1*

Length: 1917 nts (nucleotides)

RNA binding protein

Functional category: Translation, ribosomal structure, and biogenesis

Genus/species: *P. torridus*, *S. islandicus*, *M. sedula*, *Saccharolobus solfataricus*

4 Genetic Stability of Thermo-Alkaliphilic Microbes

In general, thermophilic organisms can be defined as organisms that thrive at an optimum temperature range of 45–80 °C. This temperature range is again classified into three categories. The organism that optimally grows at 80 °C and above is termed hyper-thermophilic (Stetter 1996), extreme thermophilic organisms thrive between 65 °C and 80 °C, and moderately thermophiles grow best at 45–65 °C (Wagner and Wiegel 2008).

On the other hand, microbes can be classified into two categories based on pH; that is, organisms growing at pH 7 are termed alkali-tolerant (Wang et al. 2008), and organisms growing at pH 10 and above are known as alkaliphilic. Thermo-alkaliphilic or alkali-thermophiles are a class of microbes that possess the identities of two different extremophilic organisms. In simple terms, thermo-alkaliphilic microbes can be described as organisms that can thrive optimally at a temperature of 50 °C and above in addition to a pH of 8.5 and above (Kevbrin et al. 2004). This polyextremophile can be found in both *Archaea* and *Bacteria*. These prokaryotic organisms are capable of surviving in multiple harsh conditions as they are facilitated by diversity in their genetic makeup. In order to understand the activity of these organisms, it is important to find the connection between geographical distribution, taxonomic diversity, and the variation of adaptation.

This group of polyextremophiles is majorly found in places ranging from extremely hot places such as volcanic sites to alkaline hydrothermal vents and alkaline lakes, including Rehai Geothermal Field (China) and Lake Bogoria (Africa) (Oshima and Moriya 2008). Thriving in these extreme conditions requires various modifications in the composition of the cell membrane and cell wall, thereby minimizing the protons' and cations' permeability. From the earlier studies, it was evident that these organisms are not only found in thermo-alkaline environments but also isolated from the garden soil. To address this problem, it is crucial to characterize these species from different environments. The special attribute found in all these thermos-alkaliphiles is an excellent source of thermos-alkali stable hydrolases. This attribute facilitates their survivability and plays an important role in the biotechnology sector (Kumar and Satyanarayana 2011). In this diverse range of extreme conditions, these polyextremophiles have various abilities to survive due to the presence of unique genetic makeup. It can be articulated by saying that the earth's environmental conditions are dependent on the activity of the bacteria in their ecological niches. The study, in connection with geographical distribution and taxonomic diversity, is beneficial to understand these organisms' survival strategies along with the evolutionary processes. Both *Bacteria* and *Archaea* can be considered in the polyextremophilic group of alkali-thermophiles. The most thermo-alkaliphilic organism is *Clostridium paradoxum*, an anaerobic bacterium that grows