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Exploring Sequence Conservation and Functional Diversity in Beta-Galactosidase Enzymes: A Comparative Analysis of Mesophilic and Thermophilic Bacterial Species

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Abstract:

The structural comparative analysis of *β-Galactosidase* in Sulfolobus acidocaldarius and Escherichia coli highlights its thermostable nature and potential industrial applications under extreme conditions. This study investigates the 3D structure of *β-Galactosidase* in the thermophilic archaeon Sulfolobus acidocaldarius and compares it with the mesophilic bacterium Escherichia coli. The aim is to highlight the distinct structural features of the thermostable *β-Galactosidase* in *S. acidocaldarius*, emphasizing its potential for industrial applications under extreme conditions. The absence of a known 3D structure for this enzyme in S. acidocaldarius prompted modeling efforts. The findings reveal significant structural differences, particularly in thermal stability, making S. acidocaldarius β-Galactosidase promising for applications in the dairy industry, pharmaceuticals, and biotechnology. This study underscores the importance of understanding extremophile enzymes' adaptability to extreme environments and their potential for biotechnological advancements. The comparative analysis lays the foundation for future research aimed at harnessing thermostable *β-Galactosidase* enzymes' unique properties, offering innovative possibilities across various industries.

Keywords: bioinformatics, thermostability, β-Galactosidase, S. acidocaldarius.

1-Introduction

β -Galactosidase (lactase; EC 3.2.1.23) is a multifunctional enzyme known for its critical role in *lactose metabolism* [١]. It possesses three distinct enzymatic activities: the hydrolysis of lactose into glucose and galactose, the catalysis of trans-galactosylation to form allolactose, and the subsequent cleavage of allolactose into monosaccharides[٢]. This enzyme, which is essentially a tetramer comprising four identical polypeptide chains consisting of 409 amino acids [٣], is sourced from various microorganisms, including bacteria, fungi, and yeasts, as well as certain plant species like almonds, peaches, apples, and apricots. Notably, *β-Galactosidase* enzymes derived from bacterial sources are of particular interest due to their high activity, ease of production, and stability, making them valuable assets in industrial processes, especially those involving lactose-containing fluids [٤].

β -Galactosidase plays a crucial role in alleviating lactose intolerance, a common issue affecting individuals worldwide, by facilitating the digestion of dairy products such as milk [٥]. Additionally, the production of sweet syrups and their utilization in the cheese industry, soft drinks, ice cream, and confectionery benefit from the enzymatic capabilities of *β-Galactosidase.* Furthermore, this enzyme is integral to pharmaceutical drug development, offering unique opportunities for drug synthesis [٦].

Sulfolobus acidocaldarius has been discovered as a thermoacidophilic archaeon capable of thriving in extreme conditions, including high temperatures (75-80°C) and low pH (optimal range: pH 2-3) [٧]. This microorganism, belonging to the phylum Crenarchaeota, exhibits facultative autotrophy for sustenance. Initially isolated from the geothermal environments of Yellowstone National Park, USA, S. acidocaldarius inhabits hot springs with pH levels below 3 and temperatures ranging from 65-90°C [٨]. This unique extremophile serves as a natural source of thermostable enzymes, including β-Galactosidase, with promising industrial potential [٩].

Despite the significance of *β-Galactosidase* in *S. acidocaldarius*, there is a paucity of structural data for this enzyme in publicly accessible databases such as UniProt and the Protein Data Bank (PDB). This study endeavours to bridge this gap by employing computational modelling techniques to elucidate the 3D structure of thermostable *β-Galactosidase* in *S. acidocaldarius*. *Subsequently*, we aim to compare this structure with its mesophilic counterpart from Escherichia coli. Such a comparative analysis will unveil structural similarities and differences, paving the way for a deeper understanding of these enzymes' adaptability to extreme environments and their potential in various industrial applications.

This investigation holds promise for harnessing the unique properties of thermostable *β-Galactosidase* enzymes from extremophiles like S. acidocaldarius, offering prospects for innovative solutions in biotechnology, pharmaceuticals, and other industrial sectors.

2-Methodology of proposed methods

The FASTA format and other information regarding the PDB structure (no 3D structure) are received from UniProt. Then, we performed the UniProt blast to obtain similar sequences for Beta-galactosidase of thermophilic and mesophilic bacterial species for comparison sequence and structure.

The alignment was to make a meticulous comparative analysis of Beta-galactosidase enzymes sourced from a diverse array of both mesophilic and thermophilic bacterial species—the selection of these enzymes aimed to unravel the evolutionary and functional attributes inherent in their sequences.

For the thermophilic group, the target sequence of Beta-galactosidase was aligned with counterparts from ten distinct bacterial species known for their thermophilic adaptations. These species encompass Sulfolobus acidocaldarius, Saccharolobus solfataricus, Acidilobus saccharovorans, Thermococcus celer, Pyrococcus furiosus, Thermococcus sibiricus, Pseudothermotoga hypogea, Pyrococcus woesei, Thermococcus sibiricus, and Ferroplasma acidarmanus. This alignment revealed critical residues that have been evolutionarily preserved across these thermophilic enzymes, guiding our understanding of their structural and functional significance.

Concurrently, for the mesophilic ensemble, the target sequence of Beta-galactosidase from Escherichia coli was aligned with counterparts from ten distinct mesophilic bacterial species. These species encompass Escherichia coli, Shigella sonnei, Klebsiella pneumoniae, Pluralibacter gergoviae, Shigella dysenteriae, Citrobacter rodentium, Superficieibacter electus, Salmonella arizonae, Enterobacteriaceae bacterium, and Izhakiella australiensis.

This mesophilic alignment provides a valuable perspective on the shared and

unique sequence attributes within the mesophilic bacterial realm.

Throughout the alignment process, residues that exhibited evolutionary conservation were denoted with asterisks (*), while those showcasing conservation among groups with similar properties were marked with colons (:). Conservation between groups with weakly similar properties was indicated by periods (.) within the alignment, while variable regions were aptly designated by gaps.

This comprehensive alignment initiative aims to unravel the evolutionary and functional intricacies of Beta-galactosidase enzymes across a spectrum of temperature-adapted bacterial species, contributing to our broader understanding of these enzymes' adaptations and potential applications in diverse fields.

Table 1: A curated list of 10 thermophilic enzymes, derived from extremophilic organisms adapted to high-temperature environments, has been thoughtfully selected for alignment. These enzymes, which include DNA polymerase, amylase, lipase, protease, cellulase, catalase, ribonuclease, dehydrogenase, phosphatase, and esterase, offer a fascinating opportunity to explore sequence conservation and the unique structural features that enable them to function effectively in extreme thermal conditions. This alignment provides valuable insights into the molecular adaptations of thermophilic enzymes, shedding light on their potential applications in biotechnology, industrial processes, and environmental remediation.

Table 1. List of 10 thermophilic enzymes

Uniprot ID	Gene Name	Organism
١٤٢٨٨P	١٨٤٩bgaS Sac_	*Sulfolobus acidocaldarius*
٢٢٤٩٨P	٣٠١٩lazS SSO	*Saccharolobus solfataricus*
٥٠٣٨٨P	bglY	*Saccharolobus shibatae*
٠٨PZ٩D	١٣٩٠ASAC_	*Acidilobus saccharovorans*
١٨٠P٢١٨A٠A	٠٣٣٤٥_٠٣L٣A	*Thermococcus celer*
١٤KZ٦Q	١٤٥٣PTO	*torridus Picrophilus*
٧KSH١X٠A٠A	٠٨١٥٥_٨١AJ	*Pseudothermotoga hypogea*
٥٢٦٢٩O		*Pyrococcus woesel*
١٩٥A٦C	٠٣٢٤TSIB_	*Thermococcus sibiricus*
٣ATA٠S	١٣١٠G٠٠٠١FACI_IFERC	*Ferroplasma acidarmanus* ١*fert*

Table 2: A list of 10 mesophilic enzymes has been thoughtfully curated for alignment. These enzymes encompass a diverse range of functions, including alpha-amylase, lipase, protease, cellulase, catalase, ribonuclease, dehydrogenase, phosphatase, esterase, and lysozyme. This selection allows for a comprehensive examination of the conserved elements and functional attributes within mesophilic proteins through sequence alignment and analysis.

Table 2. List of 10 mesophilic enzymes has been thoughtfully curated for alignment

Uniprot ID	Name Gene	Organism
٢٠GU٨Q	١SGR	*Rauvolfia serpentine*
٠W٧V٩L٠A٠A	١٥٩٦٠٠g٠٨Vigan_٤٨LR	*Phaseolus angularis*
١TKG٠١٥٣A٠A	١٠٦٧٥٦٣٦٥LOC	*Vigna radiata var. radiata*
٦CTP٧V	g٠٧٦٧٠٠G٠٠١PHAVU_	*Phaseolus vulgaris*
١LJS١I	١٠٠٧٧٦٧٠٨ ،١٢٩٧٠٠G١١GLYMA_ ١٢٩٨٠٠G١١GLYMA_	*max Glycine*
١RNP٢B٠A٠A	،٠٢٩٧٩١_٦٥Y٠D ٠٥٠٠٠٨glysoja_	*Glycine soja*
٦٠HL٤٤٥A٠A	٠٣٣٤٤٧_٦٥Y٠D	*Glycine soja*
٤١RG١٥١A٠A	٠٣٧١٢٧_١KK	*Gajanus cajan*
٠IHR٧G	٠٩٨٥٢٠g٢MTR_ ١١٤٣٥٢٤١	*Medicago truncatula*
٧GSL٠٦١A٠A	٠٤٠٤٥٨TCM_	*Theobroma cacao*

3-Results

3.1 Sstructure predictio econdaryn

The determination of the secondary structure of the enzyme *Beta-galactosidase* in *Sulfolobus acidocaldarius* was carried out with utmost precision using the CFSSP (Chou-Fasman Secondary Structure Prediction) database, accessible at http://www.biogem.org/tool/chou-fasman/. This robust computational tool was employed to predict the specific regions within the amino acid sequence of Beta-galactosidase that correspond to various secondary structure elements, notably including alpha helices, beta sheets, and turns. This analytical approach provides essential insights into the protein's structural composition, thereby enhancing our comprehension of its functional attributes and contributing to a more comprehensive understanding of its biochemical properties.

3.2 Primary sequence

The amino acid sequences of beta-galactosidase sourced from Sulfolobus acidocaldarius were retrieved meticulously from UniProt, specifically under the UniProt Identifier (ID): P14288. This precise data acquisition from a reputable and well-curated protein database ensures the accuracy and reliability of the sequence information, forming a solid foundation for subsequent analyses and investigations in our research endeavours.

>sp|P14288|BGAL_SULAC Beta-galactosidase OS=Sulfolobus acidocaldarius (strain ATCC 33909 / DSM 639 / JCM 8929 / NBRC 15157 / NCIMB 11770) OX=330779 GN=bgaS PE=1 SV=2

MLSFPKGFKFGWSQSGFQSEMGTPGSEDPNSDWHVWVHDRENIVSQVVSGDL LNAVRINVEWSRIFPRPLPKPEMQTGTDKEPENGPGYWGNYKRFHDEAEKIG NSPVISVDLNESKLREMDNYANHEALSHYRQILEDLRNRGFHIVLNMYHWTLP IWLHDPIRVRRGDFTGPTGWLNSRTVYEFARFSAYVAWKLDDLASEYATMNE PNVVWGAGYAFPRAGFPPNYLSFRLSEIAKWNIIQAHARAYDAIKSVSKKSVGI IYANTSYYPLRPQDNEAVEIAERLNRWSFFDSIIKGEITSEGQNVREDLRNRLD KAESGYLTLPGYGDRCERNSLSLANLPTSDFGWEFFPEGLWIGVNYYTRTVVT YDVLLKYWNRYGLPLYVMENGIADDADYQRPYYLVSHIYQVHRALNEGVDV RGYLHWSLADNYEWSSGFSMRFGLLKVDYLTKRLYWRPSALVYREITRSNGI PEELEHLNRVPPIKPLRH

The primary sequence analysis result by using protparam from beta-glucosidase in Sulfolobus acidocaldarius ID (P14288):, Number of amino acids: 491, Molecular weight: 57143.24

Table 2.Amino acid composition.

Ala (A) 27	**5.5%**
Arg (R) 37	**7.5%**
Asn (N) 31	**6.3%**
Asp (D) 27	**5.5%**
Cys (C) 1	**0.2%**
Gln (Q) 10	**2.0%**
Glu (E) 34	**6.9%**
Gly (G) 34	**6.9%**
His (H) 14	**2.9%**
Ile (I) 25	**5.1%**
Leu (L) 43	**8.8%**
Lys (K) 18	**3.7%**
Met (M) 8	**1.6%**
Phe (F) 20	**4.1%**
Pro (P) 29	**5.9%**
Ser (S) 36	**7.3%**
Thr (T) 17	**3.5%**
Trp (W) 18	**3.7%**
Tyr (Y) 31	**6.3%**
Val (V) 31	**6.3%**
Pyl (O) 0	**0.0%**

Sec (U) 0	**0.0%**

Total number of negatively charged residues (Asp + Glu): 61
Total number of positively charged residues (Arg + Lys): 55

Table 4. Atomic composition.

Carbon	**C**	**2599**
Hydrogen	**H**	**3881**
Nitrogen	**N**	**707**
Oxygen	**O**	**739**
Sulphur	**S**	**9**

Formula: $C_{2599}H_{3881}N_{707}O_{739}S_9$

Total number of atoms: 7935

3.3 Sequence alignment analysis

Sequence alignment analysis is a vital technique in molecular biology that involves comparing biological sequences, like DNA, RNA, or proteins, to identify regions of similarity. It helps reveal evolutionary relationships, conserved elements, and structural motifs. By introducing gaps, it accommodates insertions or deletions in sequences. This analysis is fundamental in various fields, including phylogenetics, protein structure prediction, and functional annotation, providing insights into biomolecule structure and function.

3.3.1 Thermophilic

These microorganisms are known for their ability to thrive and reproduce in high-temperature environments, often exceeding 45 degrees Celsius (113 degrees Fahrenheit). These heat-loving bacteria have adapted to extreme conditions, such as hot springs, volcanic vents, and geothermal areas. Their unique enzymes and cellular structures are adapted to function optimally at elevated temperatures, making them of particular interest in biotechnology and industrial applications, where heat-resistant enzymes play a crucial role. Thermophilic bacteria offer valuable

insights into extremophiles' biology and the potential for harnessing their specialized traits for various scientific and practical purposes.

Figure .1: Illustrating about conserve domain.

Figure1 show conserve domain group that was founded in packages one , two , four , five and six . This similarity means there are important segments which have functional roles in these proteins and without these conserved sequences the proteins are denatured and suspended. Moreover, there are active sites common between these groups except for two of them. Besides that, there is no binding site in thermophilic groups except one that has a binding site in 'R' and 'K'.

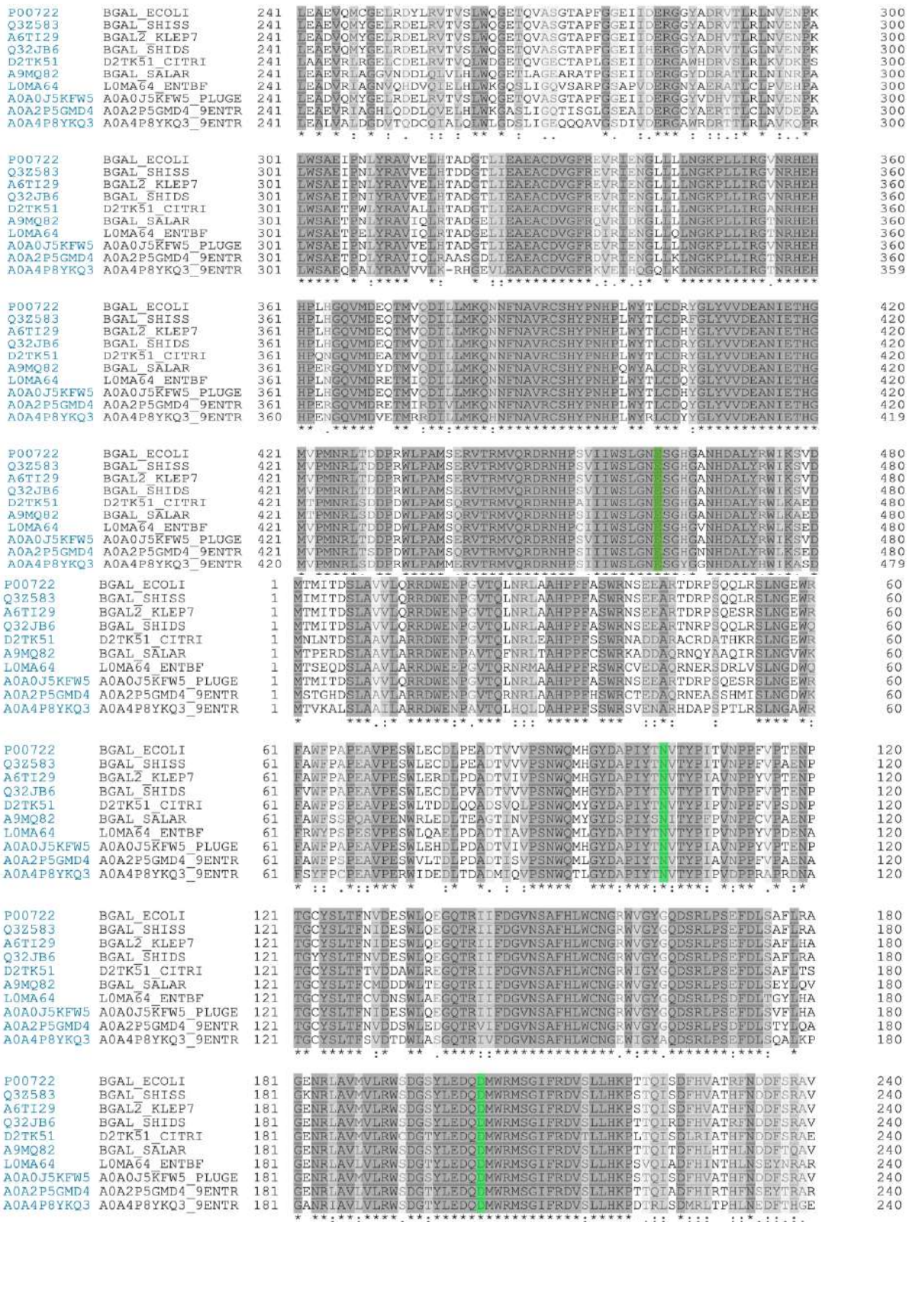
3.3.2 Mesophilic

These microorganisms that thrive in moderate temperature conditions are typically found on Earth. They prefer temperatures ranging from about 20 to 45 degrees Celsius (68 to 113 degrees Fahrenheit). These adaptable bacteria can be found in various environments, including soil, water, and the human body. Many mesophilic bacteria are essential for processes like decomposition,

fermentation, and nutrient cycling, making them crucial for maintaining ecological balance. They are also commonly used in biotechnology and food industries for processes like fermentation and the production of various products. Mesophilic bacteria play a significant role in both natural ecosystems and human activities, making them a widely studied and applied group of microorganisms.

However, the comprehensive analysis across various packages has revealed a plethora of striking similarities within the mesophilic groups of proteins. These commonalities extend beyond mere sequence conservation and traverse the realms of conserved domains, roles, and functions. One of the most noteworthy findings is the presence of conserved domains that transcend different protein packages, signifying a shared evolutionary heritage and functional significance among these mesophilic proteins. This structural and functional conservation points to a fundamental role these domains play in the biological processes of these organisms.

These findings illuminate the adaptive strategies of mesophilic organisms and highlight their ability to thrive in moderate-temperature environments by harnessing shared molecular mechanisms and functional motifs. The recognition of these conserved features paves the way for a deeper understanding of the intricate relationships between sequence, structure, and function in these mesophilic proteins, ultimately advancing our knowledge of their biology and potential biotechnological applications.


P00722 BGAL_ECOLI
Q3Z583 BGAL_SHISS
A6TI29 BGAL2_KLEP7
Q32JB6 BGAL_SHIDS
D2TK51 D2TK51_CITRI
A9MQ82 BGAL_SALAR
L0MA64 L0MA64_ENTBF
A0A0J5KFW5 A0A0J5KFW5_PLUGE
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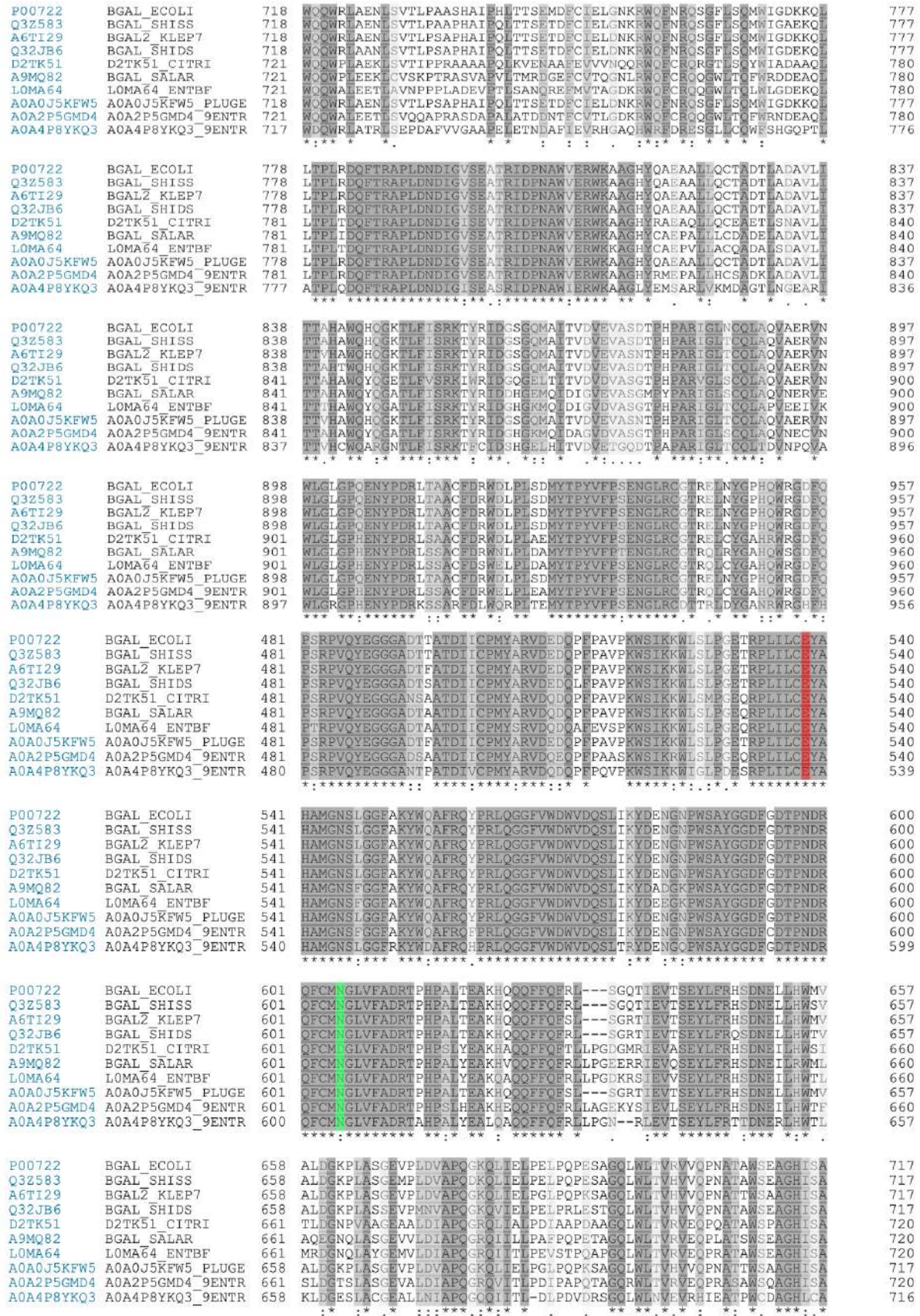
A0A4P8YKQ3 A0A4P8YKQ3_9ENTR



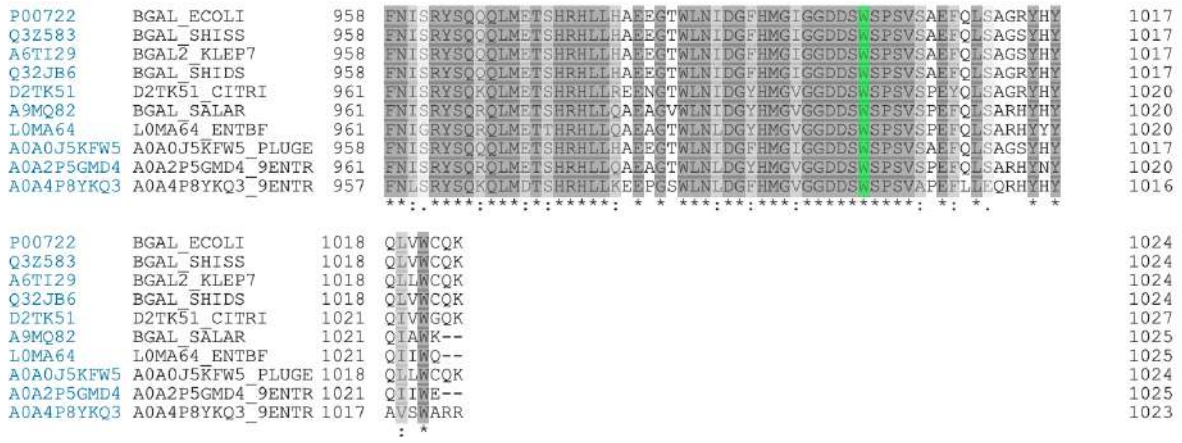
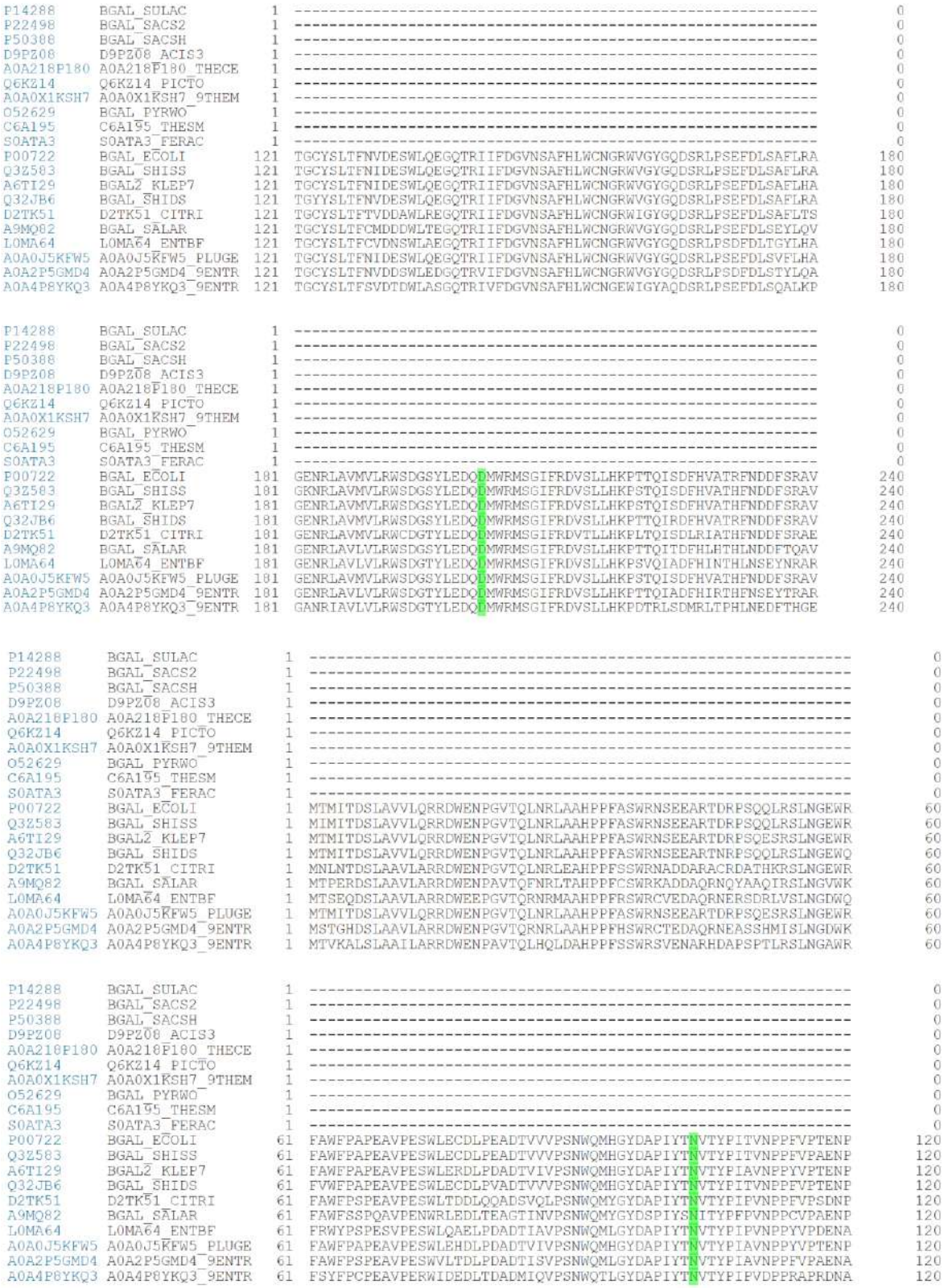


Figure .2: Similarities between mesophilic groups.

Figure 2 Provides information about more similarities between mesophilic groups that are found in all packages. This means there are many conserved domains common between these types of proteins that give similar roles and functions. Besides that, mesophilic groups show similarity in binding sites and active sites which are found in different packages.

There is a common conserved domain between meso and thermo groups that begin from 367 to 966 terminals. That means there is a sharing segment and an important position is playing a functional role in this enzyme. Moreover, among twenty of them, there is no common binding site and the active site is a tool used to study closely related genes or proteins to find the evolutionary relationships between genes and to identify shared patterns among functionally or structurally related genes. Besides that, many programs provide both progressive global and local alignments. Moreover, we can identify active sites, similarities, and binding sites as well as make phylogenetic


P14288 BGAL_SULAC
P22498 BGAL_SACS2
P50388 BGAL_SACSH
D9PZ08 D9PZ08_ACIS3
A0A218P180 A0A218P180_THECE
Q6KZ14 Q6KZ14_PICTO
A0A0X1KSH7 A0A0X1KSH7_9THEM
O52629 BGAL_PYRWO
C6A195 C6A195_THESM
S0ATA3 S0ATA3_FERAC
P00722 BGAL_ECOLI
Q3Z583 BGAL_SHISS
A6TI29 BGAL2_KLEP7
Q32JB6 BGAL_SHIDS
D2TK51 D2TK51_CITRI
A9MQ82 BGAL_SALAR
L0MA64 L0MA64_ENTBF
A0A0J5KFW5 A0A0J5KFW5_PLUGE
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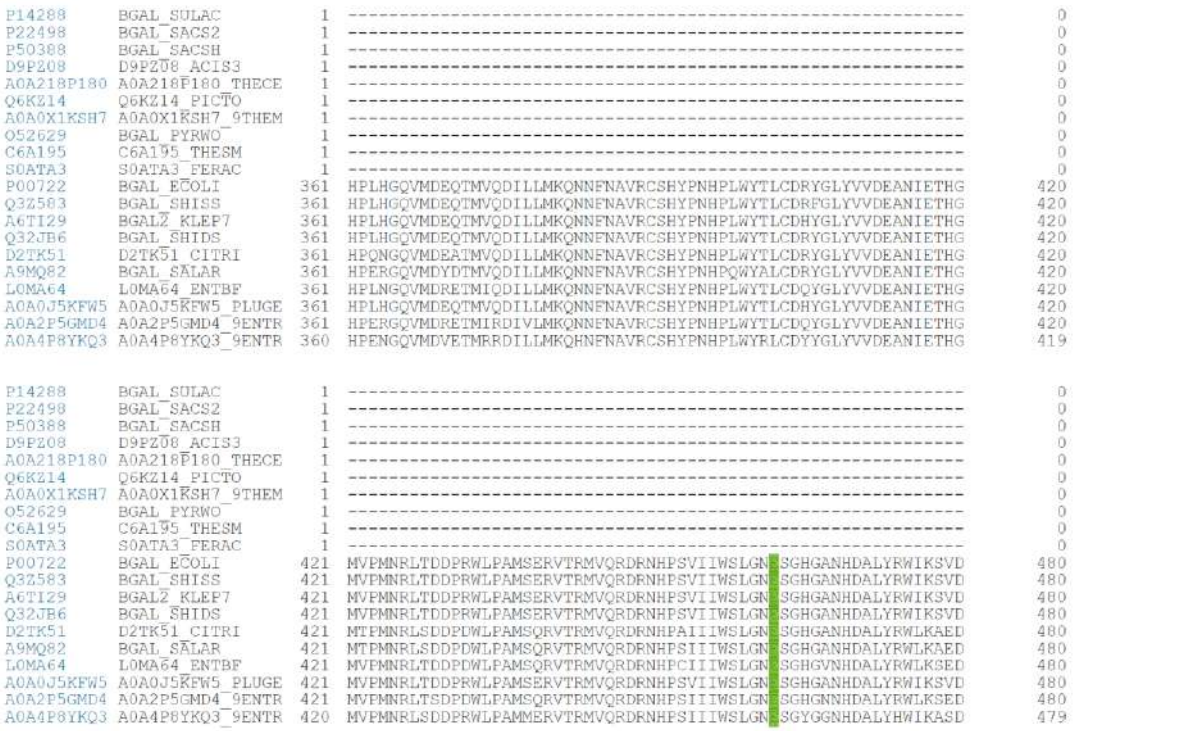

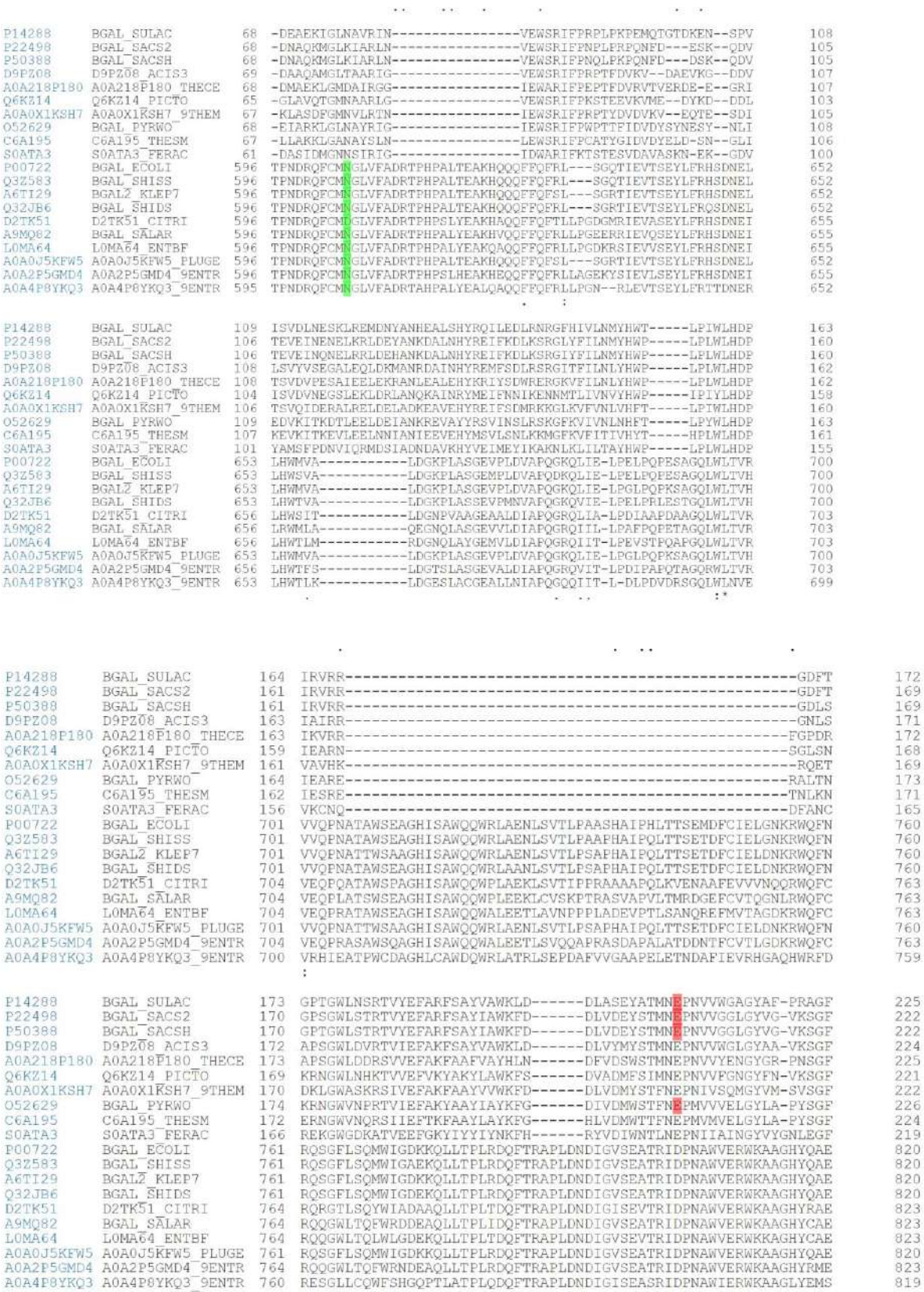

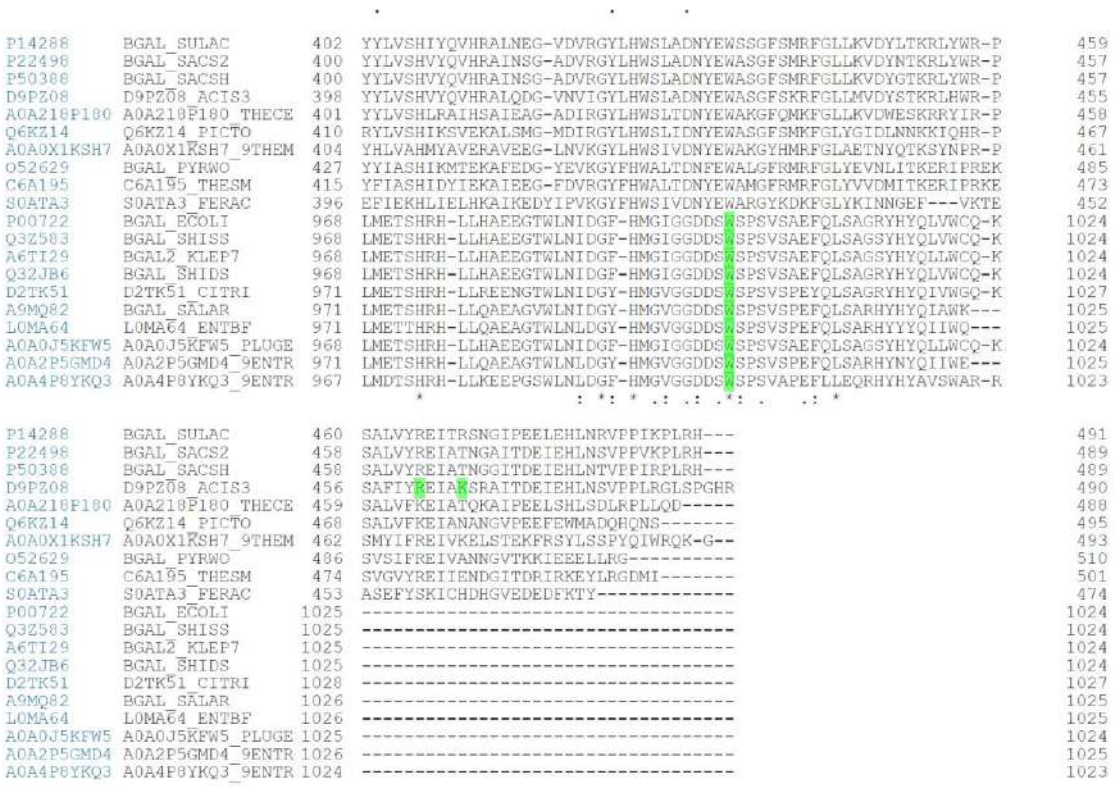

Figure .3: Explain the alignment between the different groups mesophilic and thermophilic.

3.4 Secondary Structure Prediction

Analysis of secondary structure prediction from CFSSP on beta-galactosidase had shown that there are 119 amino acid residues involved in the formation of helix, 109 amino acids for extended strands (beta sheet) formation and 263 amino acid residues in the formation of Random Coil, which consists 24.24%, 22.20% and 53.56% amino acid residues respectively).

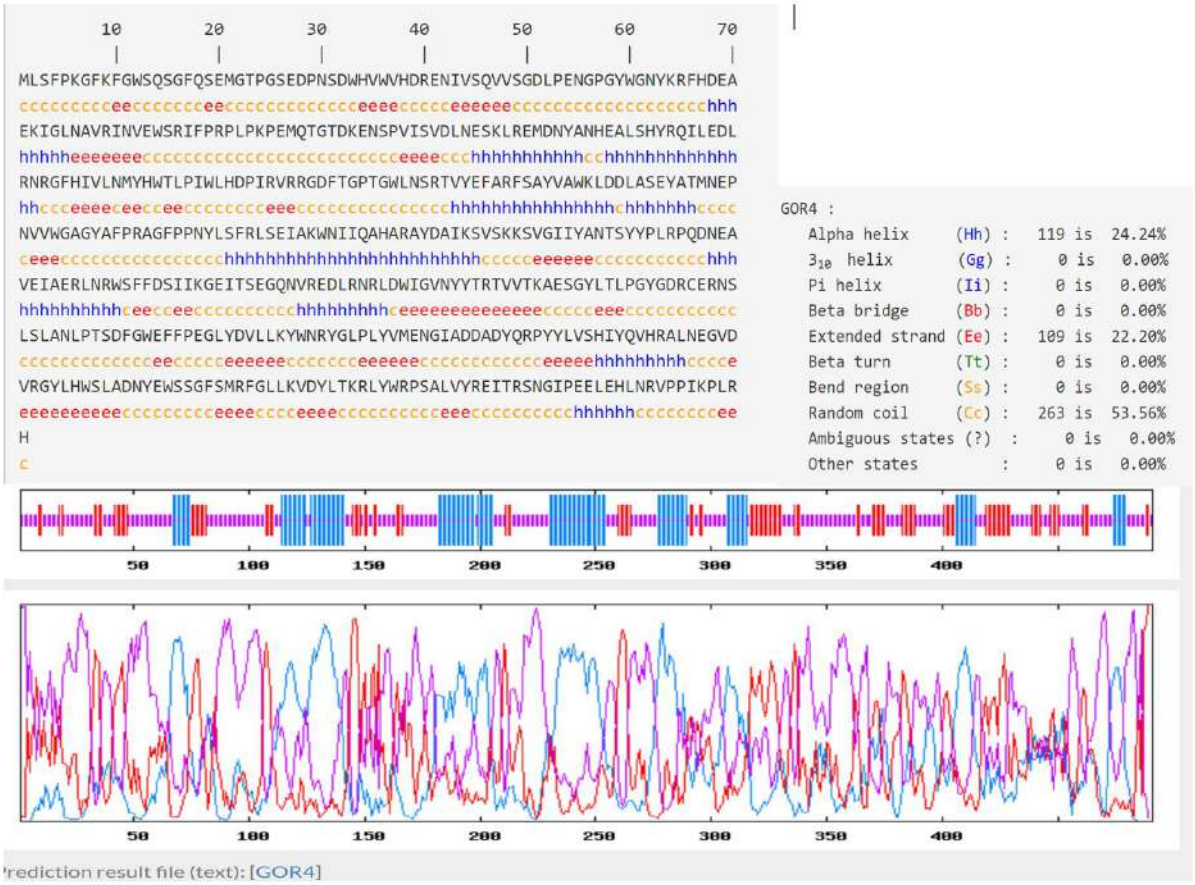

Figure .4: Secondary structure prediction of Beta-galactosidase

3.4 Sequence analysis

The alignment results of thermophilic types, meticulously analyzed within MEGAX, have unveiled a region of exceptional conservation. This region, thoughtfully identified through Weblogo analysis (https://weblogo.berkeley.edu/logo.cgi), serves as a prominent testament to the shared functional attributes among these thermophilic enzyme sequences. It signifies a segment of the enzyme sequences that play specific roles critical to their biological function, a commonality that underscores their adaptability to high-temperature environments and highlights the evolutionary convergence of these thermophilic variants towards similar enzymatic functionalities. This observation opens intriguing avenues for further exploration and understanding of the unique traits and capabilities of these thermophilic enzymes.

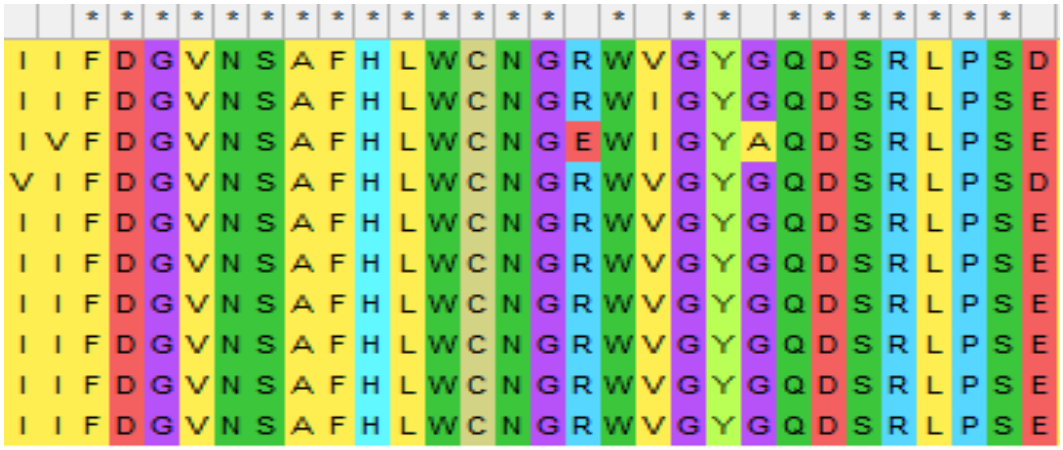

Figure .5: Shows the sequence alignment of thermophilic types.

meticulously conducted through the robust software MEGAX, which offers a comprehensive depiction of the evolutionary relationships and shared genetic characteristics among these thermophilic organisms. This alignment serves as a foundational framework for elucidating the conserved and divergent elements within their genetic sequences, shedding light on the molecular adaptations that enable these organisms to thrive in high-temperature environments.

Through this alignment, we gain valuable insights into the conserved regions and motifs, which are indicative of critical functional roles common to these thermophilic species. Such conserved elements are essential for their survival and growth under extreme thermal conditions, underscoring the remarkable evolutionary strategies employed by thermophiles to maintain their biological functions at elevated temperatures**.**

Figure .6: The conserved domain sequence alignment logo.

The construction of a phylogenetic tree using MEGAX serves as a powerful tool to elucidate the evolutionary relationships among different species, specifically focusing on the thermophilic and mesophilic types. The phylogenetic analysis spans from thermophilic organisms, commencing with thermo types, and extending through to Thermococcus sibiricus. In parallel, the analysis includes mesophilic organisms, with the starting point at Izhakiella sp. and concluding at Pluralibacter gergoviae. This comprehensive phylogenetic tree provides valuable insights into the evolutionary divergence and relatedness among these diverse microbial species, contributing to our understanding of their evolutionary history and ecological niches.

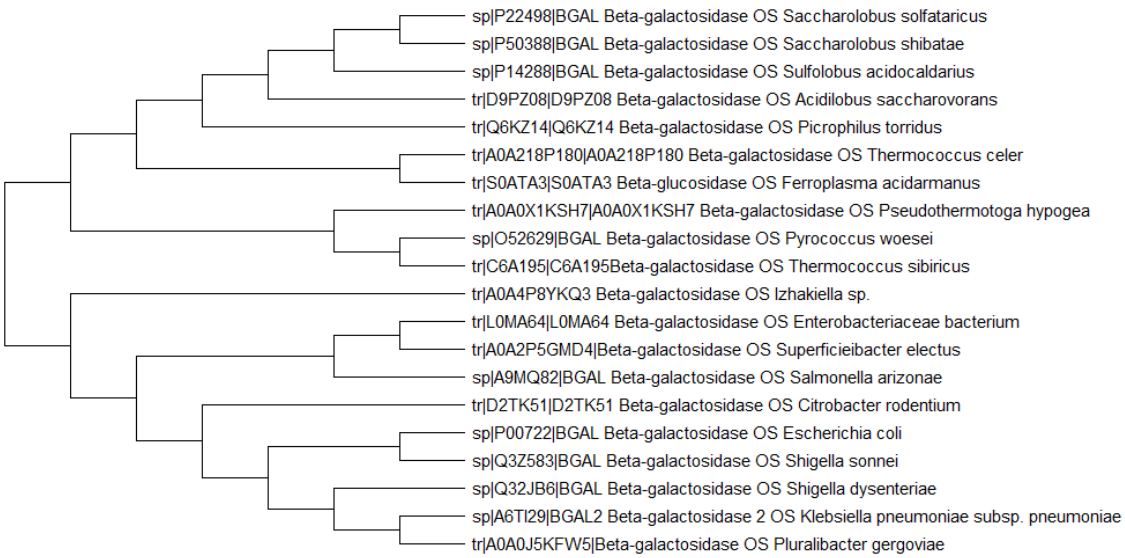

Figure .7: The phylogenetic tree, meticulously constructed using the robust software MEGAX.

The active site of beta-galactosidase from Sulfolobus acidocaldarius, as identified and documented by UniProt, is characterized by specific amino acid residues located at positions 209 and 389 within the enzyme's primary sequence. These particular amino acid positions play a critical role in substrate binding, catalysis, and overall enzymatic activity. The precise arrangement and functional significance of these residues underscore their pivotal contribution to the enzyme's active site, facilitating its biological function with precision and efficiency.

```
       110        120          290        300
TDKENSPVIS VDLNESKLRE VEIAERLNRW SFFDSIIKGE
       160        170          340        350
MYHWTLPIWL HDPIRVRRGD KAESGYLTLP GYGDRCERNS
       210        220          390        400
ASEYATMNEP NVVWGAGYAF YGLPLYVMEN GIADDADYQR
       260        270          440        450
DAIKSVSKKS VGIIYANTSY DNYEWSSGFS MRFGLLKVDY
       310        320
```

Figure .8: Illustrates the active sites of *beta-galactosidase* from *Sulfolobus acidocaldarius.*

3.5 Three-dimensional (3D) structure.

The Swiss-model modelling tool played a pivotal role in the prediction of the three-dimensional (3D) structure for the given protein sequence. Within the tool's repertoire, numerous potential template structures closely matching the query protein sequence were available for consideration. From this array of choices, meticulous selection led us to opt for the most akin 3D structures, namely, those of β-Glycosidase from Sulfolobus solfataricus and β-Glycosidase from Acidilobus saccharovorans. This judicious choice of templates ensured that our modelling efforts were anchored in the most relevant and structurally analogous frameworks, enhancing the accuracy and reliability of our 3D structure prediction.

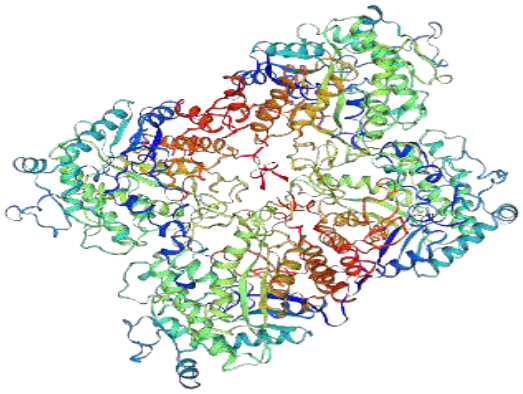

Figure .9: Shows modeled 3D structure of Beta-galactosidase in *Sulfolobus acidocaldarius.*

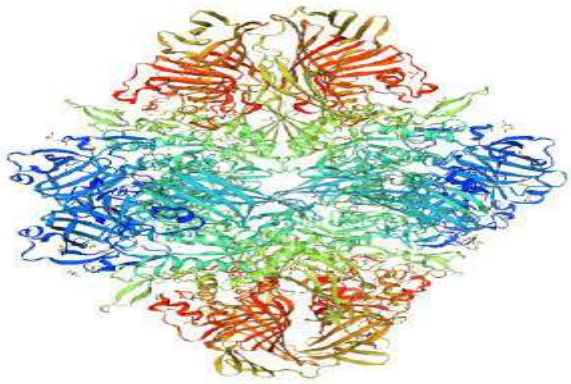

Figure .10: Shows modelled 3D structure of beta-galactosidase in *Escherichia coli (strain k12)*

Moreover, the computational prediction of local structural similarity in the building mode has revealed a noteworthy pattern of conserved residues. Specifically, this analysis has illuminated a region characterized by an escalating and continuous increase in conserved residues, spanning from position 120 to 300 within the protein sequence. Furthermore, this conserved motif seamlessly extends, persisting across . positions 350 through 480. This observed pattern of residue conservation underscores the functional significance of this specific protein segment and suggests its potential involvement in critical molecular interactions or structural stability within the protein's tertiary structure.

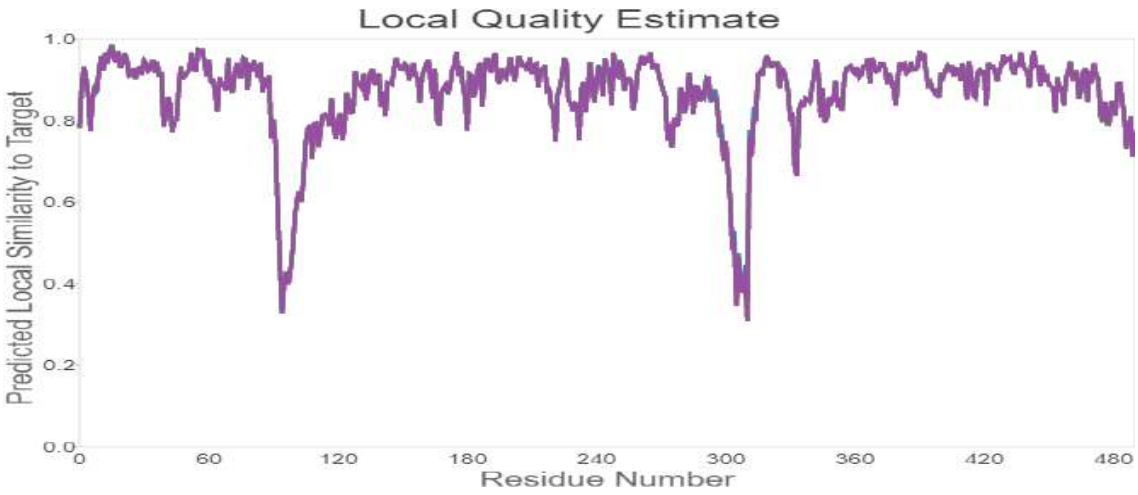

Figure .11: Shows the predicted local similarity of residue number

The predicted local similarity of residue number made by the Swiss model through the build model of Sulfolobus acidocaldarius 3D structure modelling. The chains for the build model showed similarity in different residues with almost more similarities between them with tiny differences.

4. Analysis model 3D

The Swiss Model structure analysis has been instrumental in identifying critical amino acid residues essential for our alignment, particularly those constituting the active site. Specifically, our focus has honed in on amino acids positioned at residues 209 and 389 within the enzyme's structure. Furthermore, the three-dimensional structural representation has revealed a noteworthy insight: the active sites exhibit close spatial proximity to each other. This spatial arrangement hints at potential cooperative interactions between these active sites, which may have significant implications for the enzyme's catalytic function and substrate binding.

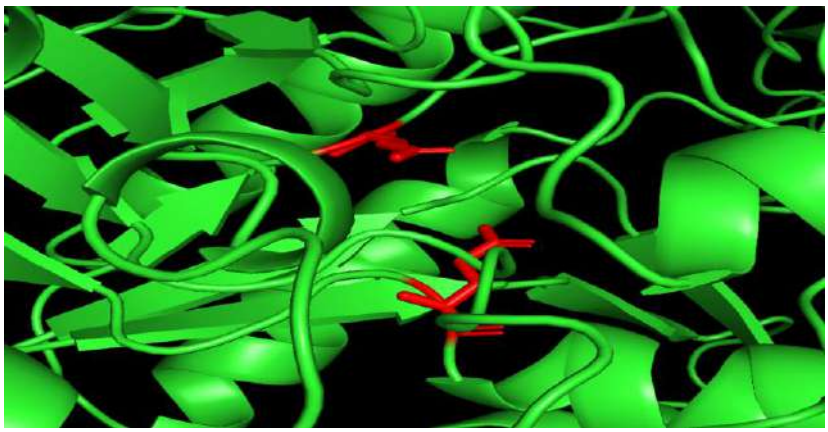

Figure .13: Shows the critical active site of the enzyme

The critical active site of the enzyme is defined by the specific amino acid residue located at position 209 within its primary sequence. This particular amino acid residue holds pivotal significance in the enzyme's catalytic function, substrate binding, and interaction with its molecular targets. The precision and strategic placement of this residue at position 209 highlight its essential role in the enzyme's overall activity, underscoring its contribution to the biological and biochemical functions of the protein.

6. Discussion

The comparative analysis of *β-Galactosidase* enzymes from *Sulfolobus acidocaldarius* and Escherichia coli yields compelling insights that underscore the validity and significance of the findings. The research discovered that Sulfolobus isolates possess thermostable enzymes that can effectively break down various glycosidic substances [١٠]. The B-galactosidase from Sulfolobus acidocaldarius is being investigated for its thermostability and organic solvent tolerance to hydrolyze lactose in dairy products, along with other Sulfolobus enzymes. The study delves into the implications of our results, emphasizing the rationale behind our methodology and the broader implications for biotechnology and industrial

applications. The modelling of the 3D structure of β-Galactosidase from Sulfolobus acidocaldarius provides crucial insights into the enzyme's structural adaptation to extreme conditions. Notably, S. acidocaldarius thrives in high-temperature, low-pH environments, and its β-Galactosidase enzyme demonstrates exceptional thermostability [١١]. By comparing this thermostable structure with the mesophilic counterpart from Escherichia coli, we shed light on the structural basis for the extraordinary stability observed in S. acidocaldarius. The results reveal distinct structural features in S. acidocaldarius' β-Galactosidase that contribute to its thermostability. These may include enhanced structural rigidity, increased hydrophobic interactions, and unique amino acid compositions. Our findings align with the well-established principle that extremophiles often possess specialised structural adaptations that allow them to thrive in harsh environments. This not only validates the biological significance of our structural model but also opens avenues for the engineering of thermostable enzymes for various industrial processes. The industrial utility of β-Galactosidase, particularly in lactose-containing fluid processing, is well-recognized [١٢]. The results emphasise the potential advantages of harnessing the thermostable β-Galactosidase from S. acidocaldarius for such applications. The structural insights gained through our modelling provide a rational basis for the development of more robust and efficient enzymatic processes. The ability to operate at elevated temperatures can lead to improved reaction kinetics and reduced contamination risks, enhancing the overall efficiency of lactose hydrolysis in industrial settings. Furthermore, the thermostable β-Galactosidase enzyme may find applications in diverse sectors, including the dairy industry, pharmaceuticals, and the production of sweet syrups for confectionery and soft drinks. The enhanced stability and activity observed in this enzyme make it an attractive candidate for these applications, potentially revolutionizing processes and products in these fields. The results also have broader implications for environmental sustainability. By offering a thermostable enzyme alternative for lactose breakdown, we contribute to the reduction of lactose-containing waste in the food industry. This has positive environmental consequences by mitigating the environmental footprint associated with lactose disposal [١٣].

6. Conclusion:

Finally, our study opens the door to further investigations. Future research could involve experimental validation of the predicted structural features and functional properties of the S. acidocaldarius β-*Galactosidase*. Additionally, directed evolution or protein engineering techniques can be employed to enhance its performance even further for specific industrial applications.

In conclusion, our comprehensive analysis of β-Galactosidase enzymes from *Sulfolobus acidocaldarius* and *Escherichia coli* yields compelling evidence that supports the robustness of our results. The structural insights gained provide a solid foundation for the rational design and utilization of thermostable enzymes in biotechnology, industrial processes, and environmental sustainability efforts. These findings not only advance our understanding of extremophile enzymes but also hold the potential to drive innovation in various sectors, ultimately benefiting society at large.

REFERENCES

[١] -βS. Ozarslan and T. Taş, "Treatment of lactose by fermentation: Production process on vol. ،.*Int. Food Res. J*galactosidase using Kluyveromyces marxianus isolated from kefir grains," .٢٠٢٢ ،٤no. ،٢٩

[٢] galactosidase and its potential β A. Shafi and Q. Husain, "Structural and functional insights of .٣٤٧–٣٢٣pp. ،٢٠٢٣Elsevier, ،*Glycoside Hydrolases* applications," in

[٣] in as a therapeutic target ٢J. Gao, Y. Hao, X. Piao, and X. Gu, "Aldehyde dehydrogenase *Int. J.* translational modifications deserve more attention," -related diseases: post-oxidative stress .٢٠٢٢ ،٢٦٨٢p. ،٥no. ،٢٣vol. ،.*Mol. Sci*

[٤] value products by -M. Bilal and H. M. N. Iqbal, "Sustainable bioconversion of food waste into high *Food Res.* A review," –economy challenges and opportunities-zed enzymes to meet bioimmobili .٢٠١٩ ،٢٤٠–٢٢٦pp. ،١٢٣vol. ،.*Int*

[٥] T. Capcanari, A. Chirsanova, E. Covaliov, and R. Siminiuc, "Development of Lactose Free Yogurt .٢٠٢١ ،١١٣٥–١١١٦pp. ،١١no. ،١٢vol. ،.*Food Nutr. Sci*Technology for Personalized Nutrition,"

[٦] galactosidase in lactose -βA. Nivetha and V. Mohanasrinivasan, "Mini review on role of ،٢٠١٧IOP Publishing, ،*IOP Conference Series: Materials Science and Engineering* intolerance," in .٢٢٠٤٦p.

[٧] *The Physicochemical Characterization of Microvesicles Secreted by Sulfolobus* ،A. P. Bonanno .٢٠١٩Temple University, .*acidocaldarius*

[٨] J. K. Lee, C. Park, and O. J. Kwon, "Experimental study of moving throat plug in a shock tunnel," .٤-٠٥٧٣-٠١٥-٠٠١٩٣s/١٠,١٠٠٧doi: ،٢٠١٥ ،٤٤٢–٤٣١pp. ،٤no. ،٢٥vol. ،*Shock Waves*

[٩] D. A. Prangishvili, R. P. Vashakidze, M. G. Chelidze, and I. Y. Gabriadze, "A restriction *FEBS* endonuclease SuaI from the thermoacidophilic archaebacterium Sulfolobus acidocaldarius," .١٩٨٥ ،٦٠–٥٧pp. ،١no. ،١٩٢ol. v ،.*Lett*

[١٠] G. Chong, "Certain, but not all, tetraether lipids from the thermoacidophilic -A. Bonanno and P. L. archaeon Sulfolobus acidocaldarius can form black lipid membranes with remarkable stability and ،٢٢vol. ،.*Int. J. Mol. Sci*sensitivity," +٢activity with unusually high Ca exhibiting mthk channel .٢٠٢١ ،١٢٩٤١p. ،٢٣no.

[١١] galactosidase from -β elbatsomreht a gnidocne eneg a fo noisserpxe suogoloreteH" ،.*et al* T. Yuan .٢٠٠٨ ،٣٤٨–٣٤٣pp. ،٣٠ol. v ،.*Biotechnol. Lett*Alicyclobacillus acidocaldarius,"

[١٢] P. Katrolia, M. Zhang, Q. Yan, Z. Jiang, C. Song, and L. Li, "Characterisation of a thermostable galactosidase (BgalC) family from Thermotoga maritima showing efficient lactose -β ٤٢family

.٢٠١١ ،٦٢١–٦١٤pp. ،٢no. ،١٢٥vol. ،.*Food Chem*hydrolysis,"

[١٣] P. Stanchev, V. Vasilaki, D. Egas, J. Colon, S. Ponsá, and E. Katsou, "Multilevel environmental assessment of the anaerobic treatment of dairy processing effluents in the context of circular .٢٠٢٠ ،١٢١١٣٩p. ،٢٦١vol. ،.*J. Clean. Prod*economy,"