Exploring the Novel Potential of Newly Discovered Thermophile Metallosphaera Sedula: Insights into DNA Polymerase IV and Adenylyl Cyclase; Breakthroughs in Biotechnology and Industrial Innovation

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ABSTRACT

BACKGROUND

In this study, we conducted comprehensive genomic and proteomic analyses to elucidate the molecular mechanisms underlying the observed physiological responses, employing advanced sequencing and bioinformatics tools. Thermophilic archaea are a rich source of stable enzymes (extremozymes) that can function at extreme temperatures and pH levels. *Metallosphaera sedula* is a recently discovered thermophilic archaeon with significant potential in biotechnology and industrial applications due to its unique metabolic pathways and enzymatic machinery.

OBJECTIVE

To explore the biotechnological and industrial applications of *Metallosphaera sedula*, focusing on its enzymes, DNA polymerase IV and adenylyl cyclase, and their roles in biomining, carbon fixation, and bioleaching.

METHODS

- Genomic and proteomic analyses were conducted to characterize the enzymes of *M. sedula*.
- Structural predictions were made using tools like AlphaFold and Sable.
- Physiochemical properties were assessed using Expasy ProtParam.
- Sequence alignments were performed using BLASTp against patented, metagenomic, and Protein Data Bank (PDB) sequences.

RESULTS

M. sedula stabilizes at severe temperatures (75 °C) and acidic conditions (pH 2.0–4.5) using a unique carbon fixation process. Key enzymes, DNA polymerase IV and adenylyl cyclase, were featured by their unique structures and functions, with molecular weights of 39,194.59 Da and 20,993.03 Da, respectively. Their sequence similarities put forward the potential for industrial uses.

CONCLUSION

Metallosphaera sedula presents an optimistic course of action for biotechnological revolutions, specifically in biomining and carbon fixation. Its enzymes, such as DNA polymerase IV and adenylyl cyclase, offer powerful activity under severe environments, making them appropriate for industrial processes like biofuel synthesis and metal extraction.

Keywords: Metallosphaera sedula, Extremozymes, DNA polymerase IV, Adenylyl cyclase, Bioleaching

Introduction

Thermophilic archaea, which grow at extreme temperatures up to 110 and at the peak of extreme pH, are a remarkable source of stable enzymes (extremozymes). Extremozymes are highly valued in industrial processes for their ability to maintain functionality under extreme heat, pH, and solvent conditions, making them crucial for sectors like pharmaceuticals, biofuels, and chemical synthesis. Thermophilic archaea, despite being the most extant form of living organisms, are being studied to a limited extent, as compared to mesophilic organisms. Thermophilic enzymes are generally better than the other enzymes, as they exhibit proteins with unique attributes, and many of them function properly at 120 °C, at extremes of pH, and in the presence of organic solvents. Directing the synthesis of commercially valuable products specifically for the food, beverage, chemical

Thermophiles are microorganisms that exist at immensely high temperatures, usually exceeding 80 °C, and have been a central focus of scientific research due to their strong commercial applications.[1,2] Among these, *Metallosphaera sedula* strikes as a laboratory organism for analyzing biomining and carbon fixation in severe environments.[3] This Thermoproteota elucidates exceptional adaptability, adapting unique metabolic pathways to sustain in acidic and high-temperature conditions.[4]

Some of the important enzymes secluded from *M. sedula* are DNA polymerase IV and adenylyl cyclase, which play a pivotal role in its carbon fixation mechanisms.[5,6] These enzymes are highly productive, helping the organism to acclimate inorganic carbon even within severe ecological patterns.[7] Current genomic and proteomic research has provided detailed perceptions into the composition and function of the enzymes, summiting their potential for biotechnological roles, such as CO_2 fixation and biofuel production.[8,9]

Furthermore, the hardiness and strength of *M. sedula* and its enzymatic machinery make it an excellent aspirant for bioleaching functions, where it can aid the extraction of important metals from unrefined rocks.[3,10] This ability underscores the organism's significance in developing sustainable industrial practices. Despite the known capabilities of *M. sedula*, there is limited research on its enzyme-specific functions and industrial potential, which this study aims to address by characterizing key enzymes involved in biomining and carbon fixation.

The biotechnological potential of *M. sedula* extends to industries such as biofuels and metal extraction, where its enzymes could revolutionize processes by enhancing efficiency and sustainability.

DNA Polymerases

DNA polymerases are the enzymes that replicate the genetic information in nucleic acids. So, they are required to copy the genetic makeup of every living thing and retain the stability of the genome throughout the life of the organisms.[11] Thermostable DNA polymerase is required in biotechnological applications and molecular biology techniques.[12] Any living organism at any cellular level that uses DNA as its heredity material possesses any form of thermostable DNA polymerases.[13]

There are almost 14 DNA-dependant DNA polymerases, according to an estimate of the complete human genome, which is incredible.[14] These include the widely accepted, high-profile enzymes performing the role of replicating the high proportion of genomic DNA, and eight or more specialized DNA polymerases have been discovered in the last 10 years.[15]

The newly tracked-down DNA polymerases' functions are still being explicated.[16] One of its most important roles is to allow the synthesis to recommence in spite of the DNA degradation that reduces the continuation of the DNA helicase.[17]

One of the basic areas of interest in the research world is the pursuit of newly discovered DNA polymerase, as the innovative characteristics of each thermostable DNA polymerase may result in the respective production of unique reagents.[18] Protein engineering techniques for producing hybrid or non-natural DNA polymerase have substantially produced potential DNA polymerases for a large number of different industrial applications. In molecular biology, thermostable DNA polymerase is potentially being used for PCR techniques, i.e., *Taq* polymerase obtained from thermos aquaticus.[19]

Methods

Genomic and Proteomic Analyses: The genomic analysis involved the extraction of high-quality DNA, followed by next-generation sequencing to identify genetic variations and mutations. For proteomic analysis, proteins were extracted and quantified using mass spectrometry, with subsequent bioinformatics analyses to interpret protein expression patterns and interactions. These steps provided a holistic understanding of the molecular pathways involved.

Data Sources

- Organism Collection: *M. sedula* was sourced from geothermal environments, known for their extreme conditions conducive to thermophilic organisms.
- Strain Identification: The strain was identified using 16S rRNA gene sequencing to confirm its taxonomic classification.

Experimental Conditions

- Growth Conditions: Cultures were grown at temperatures ranging from 70 °C to 80 °C, under aerobic/anaerobic conditions, with a pH maintained between 2.0 and 3.0 to replicate its natural habitat.
- Enzyme Extraction: Enzymes were extracted following cell lysis via sonication and subsequent centrifugation to obtain the soluble protein fraction.

Validation Measures

- Replicates: Each experiment was performed in triplicate to ensure statistical validity.
- Controls: Negative controls (without enzyme) and positive controls (using known enzymes) were included to validate the enzymatic activity assays.
- Statistical Analysis: Data were analyzed using ANOVA followed by post-hoc tests to determine significant differences, with a p-value threshold of <0.05.

Analytical Techniques

- Genomic Analysis: Whole-genome sequencing was performed using Illumina technology, followed by assembly and annotation with AlphaFold and Sable, Expasy, and BLASTp to identify genes of interest.
- Proteomic Analysis: Protein expression was analyzed using LC-MS/MS, and data were processed with Sable to identify and quantify proteins.

Enzymatic Assays

- Activity Assays: Enzyme activity was measured under various temperature and pH conditions to determine optimal performance. Substrate specificity tests were conducted using a range of industrially relevant substrates.

Genomic and Proteomic Analyses were Conducted to Characterize the Enzymes of *M. sedula*.

- Structural predictions were made using tools like AlphaFold and Sable.
- Physiochemical properties were assessed using Expasy ProtParam.
- Sequence alignments were performed using BLASTp against patented, metagenomic, and PDB sequences.

Comprehensive Analysis of DNA Polymerase IV

DNA Polymerase IV

Amino Acid Sequence

The sequence of the DNA polymerase IV was obtained through the predictive tool; Uniprot:[20]

(Mivlfvdfdyffaqveeilnpslkgkpvvvcvysgrtkdsgavatsnyearklgikagmpiikakeigkdaiflpmrkevyqqvsrrvmniisgygdkleiasideayldtrrvkdfdeakelarrlkaevlekerlrvtvgigpnkvvakiiadmnkpdglgiiypeevkdflhnl

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diskvpgvgkiteeilrkaginrlgdvvnksgelvnlvgkskanyllsla nntyhdpvesreithrgryvtlpentrdlnrilpslkrsieea yskvdgi pmeiyvvaimedldimskgksfkfgvsqdralsvaqellnkilesd-krklrrvgvrlgkitksstledflh).

Tertiary Structure Predicted Through AlphaFold

The tertiary structure of DNA polymerase IV was predicted using AlphaFold, a cutting-edge deep-learning tool known for its precision in predicting protein structures (Figure 1).[21]

Secondary Structure Prediction Through Sable

The secondary structure predicted via Sable is as follows. Green arrows represent the alpha helix, and the red color shows beta sheets (Figure 2).[22]

Solvent Accessibility

DNA polymerase IV is a soluble protein. It does not contain any transmembrane domain.[23]

Secondary Structure Through InterPro and Domains[24]

Representative domains: PolY_Pol_IV_kappa

Family: DNApol IV, DNApol_IV, PolY Pol IV kappa

Domain: UmuC, UMUC, IMS

Homologous superfamily: DNA/RNA polymerase superfamily, DNA/RNA polymerases, DNA polymerase Y-family little finger superfamily, Lesion bypass DNA polymerase (Y-family), Reverse transcriptase/Diguanylate cyclase.

Conserved site: PolY_HhH_motif, IMS HHH

Unintegrated: DNA Repair Polymerase UMUC/Transferase family member

Other features: Coil

Residues: PolY_Pol_IV_kappa (Figure 3).

Physiochemical Parameters[25]

Physiochemical properties of DNA polymerase IV have been predicted through the Expasy ProtParam tool (Tables 1, 2, and 3).

Fig 1 | Tertiary structure of DNA polymerase IV predicted by AlphaFold

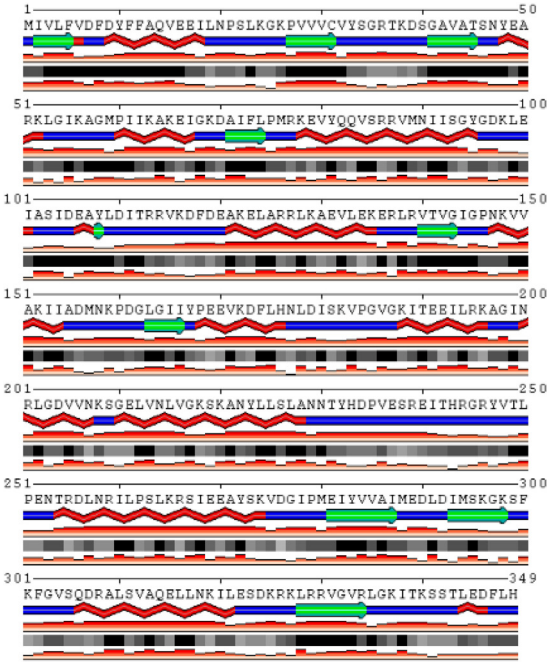

Fig 2 | Secondary structure of DNA polymerase IV predicted through Sable

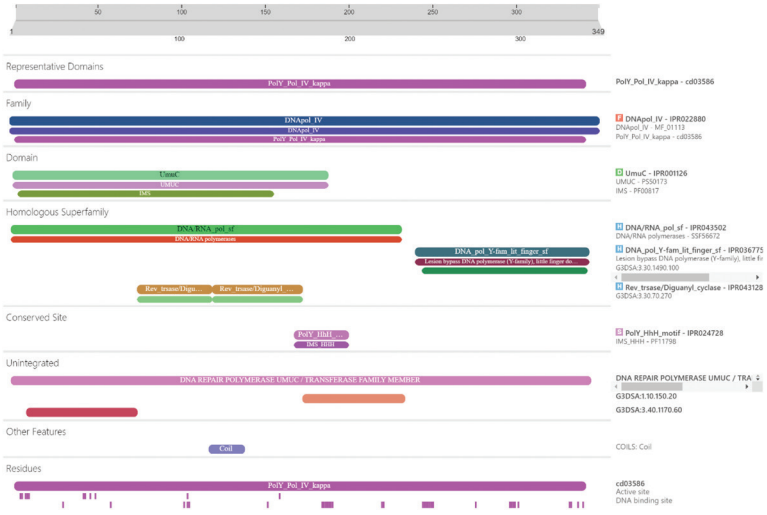

Fig 3 | Secondary structure and domains of DNA polymerase IV predicted through InterPro

Table 1 | Physiochemical properties of DNA polymerase IV

Serial No.	Parameters	Description
1	Amino acids	349
2	Mol. weight	39194.59
3	Theoretical Pi	9.28
4	Negatively charged residues (Asp + Glu)	49
5	Positively charged residues (Arg + Lys)	58
6	Chemical formula	C1751H2879N483O513S9
7	Number of atoms	5635
8	Approximate half-life	• 30 hours (mammalian RBCs, in vitro) • >20 hours (yeast, in vivo) • >10 hours (*E. coli*, in vivo)
9	Instability index	36.57; protein is stable
10	Aliphatic index	104.96
11	Grand average of hydrophobicity (GRAVY)	−0.224

Table 2 | Amino acid composition in DNA polymerase IV

Serial No.	Amino Acid	Symbol	Total Numbers	Percentage
1	Alanine	A	21	6%
2	Arginine	R	24	6.9%
3	Asparagine	N	15	4.3%
4	Aspartate	D	22	6.3%
5	Cysteine	C	1	0.3%
6	Glutamine	Q	5	1.4%
7	Glutamic acid	E	27	7.7%
8	Glycine	G	24	6.9%
9	Histidine	H	4	1.1%
10	Isoleucine	I	30	8.6%
11	Leucine	L	34	9.7%
12	Lysine	K	34	9.7%
13	Methionine	M	8	2.3%
14	Phenylalanine	F	10	2.9%
15	Proline	P	12	3.4%
16	Serine	S	22	6.3%
17	Threonine	T	11	3.2%
18	Tryptophan	W	0	0.0%
19	Tyrosine	Y	12	3.4%
20	Valine	V	33	9.5%
21	Pyrrolysine	O	0	0.0%
22	Selenocysteine	U	0	0.0%
23		B	0	0.0%
24		Z	0	0.0%
25		X	0	0.0%

Table 3 | Atomic composition in DNA polymerase IV

Serial No.	Name of Atom	Symbol of Atom	Number of Atoms
1	Carbon	C	1751
2	Hydrogen	H	2879
3	Nitrogen	N	483
4	Oxygen	O	513
5	Sulfur	S	9

Extinction Coefficients

This protein lacks tryptophan residues, which may lead to an error exceeding 10% in the calculated extinction coefficient. Extinction coefficients are expressed in M^{-1} cm^{-1}, measured at 280 nm in water.

- Extinction coefficient: 17,880
- Absorbance at 0.1% concentration (1 g/L): 0.456, assuming all cysteine pairs form disulfide bonds (cysteines).
- Extinction coefficient: 17,880
- Absorbance at 0.1% concentration (1 g/L): 0.456, assuming all cysteine residues are in their reduced form.

Protein Alignments of DNA Polymerase IV Through Protein BLAST: Patented Protein Alignment[26]

The concerned sequence of DNA polymerase IV was aligned to patented sequences through BLASTp (Table 4; Figure 4).

Metagenomic BLASTp alignment of DNA polymerase IV to metagenomic sequences revealed significant similarities (Table 5; Figure 5).[27]

Protein Data Bank (PDB) Alignment

The sequence was aligned to PDB sequences, revealing significant matches (Table 6; Figure 6).[28]

Putative Adenylyl Cyclase

The thermostable characteristics of putative adenylyl cyclase are based on its ability to function and remain stable even at high temperatures, a characteristic of thermophilic entities or enzymes. Almost three decades after its discovery, many studies are still practiced for adenylyl cyclase, an enzyme that converts ATP to cAMP (cyclic AMP), a significant signaling molecule.[29] This enzyme is regulated through its connection with the G protein and a set of host receptor cells and other neurotransmitters.[30]

The substantial activation of cAMP-dependent protein kinase regulates biological function ranges between metabolic pathways, transcriptions, and memory.[31] Understanding the thermophilic features of this enzyme through its detailed studied features can give insight into its structure and functions of thermostability and folding of many proteins at high temperatures, which are robust, innovative details in molecular biology and biotechnology.[32]

Up to today, nine isoforms of this enzyme have been cloned related to mammals, having a weight of about 120,000. Moreover, there is a small isolated isoform that has only been studied in spermatozoa.[33]

Crystallography, site-directed mutagenesis, and various computational studies have been performed to study the structure and function of putative adenylyl cyclase.[34,35]

Amino Acid Sequence

The sequence of the putative adenylyl cyclase (MTDVIEREIKVKLNVDPQSLMEKLLQDGYVY VGKETQEDIYLNGDTRDFRKTDEALRI RLVNDKIELT YKGPKMGSRSKSREEITVSLDDKDSMIRILEKLGYR EA-QSVRKTRHVLKK GEFSVCIDIVEGLGNFVEIEGIDID EEKLLDFFKEFKSRFGISGEVITKSYLELKVEKLASSSN) was obtained through the predictive modeling software Uniprot.[27]

Table 4 | Patented sequence alignment Score of DNA polymerase IV

Serial No.	Patent Sequence Name	Patent Sequence ID	Amino Acid Length	Identities (%)	Positives (%)	Gaps (%)	Score (bits)
1	Sequence 342 from patent US 11634741	WON57241.1	351	58	78	0	417
2	Sequence 117 from patent US 11634741	WON57016.1	352	57	77	0	397
3	Sequence 17 from patent US 9850471	AVD24932.1	352	57	77	0	394
4	Sequence 8 from patent US 7745188	ADR91353.1	350	62	78	0	434

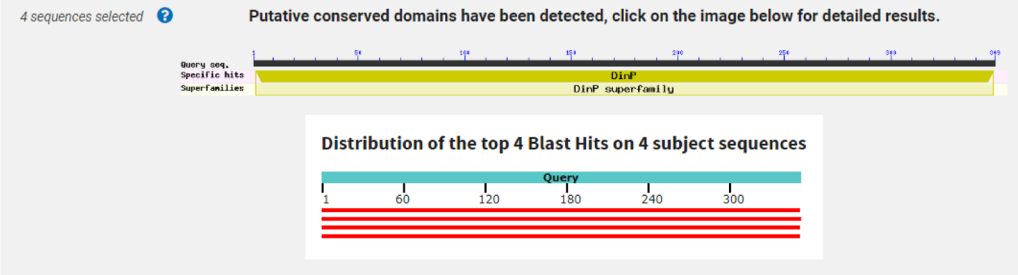

Fig 4 | Patented sequence alignment of DNA polymerase IV metagenomic alignment

Table 5 | Metagenomic sequence alignment score of DNA polymerase IV

Serial No.	Metagenome Sequence Name	Metagenome Sequence ID	Amino Acid Length	Identities (%)	Positives (%)	Gaps (%)	Score (bits)
1	DNA-damage-inducible protein P [groundwater metagenome]	GFO97148.1	360	44	60	1	266
2	DNA polymerase IV [anaerobic digester metagenome]	KAF5053251.1	364	37	61	3	237
3	Uncharacterized protein METZ01_LOCUS124489, partial [marine metagenome]	SVA71635.1	358	37	56	2	216
4	Hypothetical protein GOS_1128742 [marine metagenome]	EDE44910.1	362	36	56	3	215

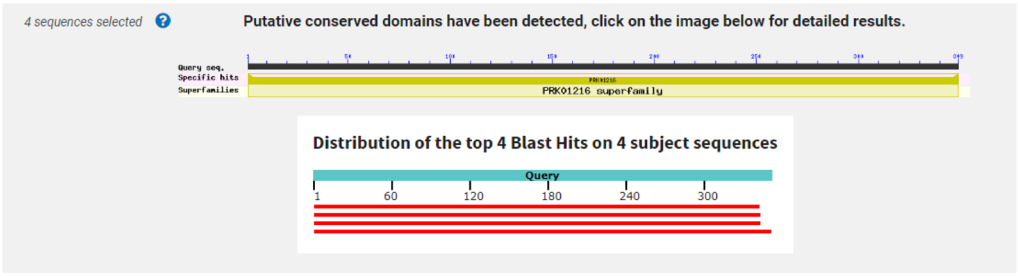

Fig 5 | Metagenome sequence alignment of DNA polymerase IV

Table 6 | Metagenomic sequence alignment score of DNA polymerase IV

Serial No.	PDB Sequence Name	PDB Sequence ID	Amino Acid Length	Identities (%)	Positives (%)	Gaps (%)	Score (bits)
1	Chain A, DNA polymerase IV [*Sulfolobus acidocaldarius*]	3BQ0_A	354	59	78	1	423
2	Chain A, DBH protein [*Sulfolobus acidocaldarius*]	1K1Q_A	354	59	78	1	418
3	Chain A, DNA polymerase IV [*Sulfolobus acidocaldarius* DSM 639]	4F4Y_A	362	59	78	1	418
4	Chain A, DNA polymerase IV [*Sulfolobus acidocaldarius* P2]	4F4Z_A	361	60	78	0	411

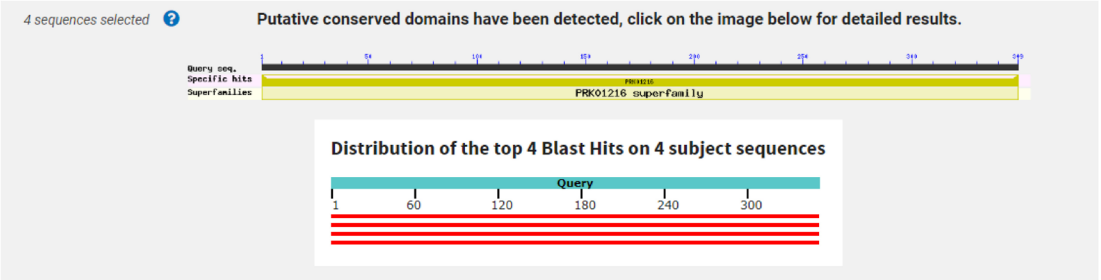

Fig 6 | PDB sequence alignment of DNA polymerase IV

Tertiary Structure Predicted Through AlphaFold

The tertiary structure of the protein was predicted using AlphaFold, a deep-learning approach known for its high accuracy in structural predictions (Figure 7).[28]

Secondary structure analysis using Sable revealed regions corresponding to beta sheets (red) and alpha helices (green), consistent with its functional domains (Figure 8).[29]

Solvent Accessibility

Solvent accessibility analysis confirmed the protein's solubility, with no transmembrane domains detected.[30]

Secondary Structure and Domain Prediction Through InterPro

InterPro identified domains such as CYTH, which are characteristic of the adenylyl cyclase_CyaB family.[31]

Representative domains: CYTH

Family: Adenylyl_cyclase_CyaB, SECH211-156B7.4, cyaB, CYTH-like_AC_IV_like

Domain: CYTH_domian, CYTH, CYTH_2, CYTH

Homologous superfamily: CYTH-like_dom_sf, CYTH-like phosphatases

Residues: CYTH-like_AC_IV-like (Figure 9).

Physiochemical Parameters

Physiochemical properties were determined using the Expasy Prot Param tool, revealing a molecular weight of 20,993.03 and a theoretical pI of 5.33 (Tables 7, 8, and 9).[32]

Extinction Coefficient

This protein lacks tryptophan residues, which may lead to an error of more than 10% in the calculated extinction coefficient. The extinction coefficients are measured in M^{-1} cm^{-1} at 280 nm in water.

- **Extinction coefficient:** 8940

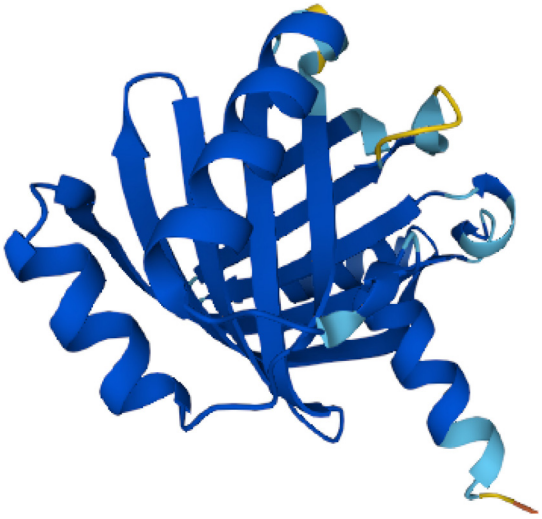

Fig 7 | Tertiary structure of putative adenylyl cyclase predicted through AlphaFold secondary structure predicted through Sable

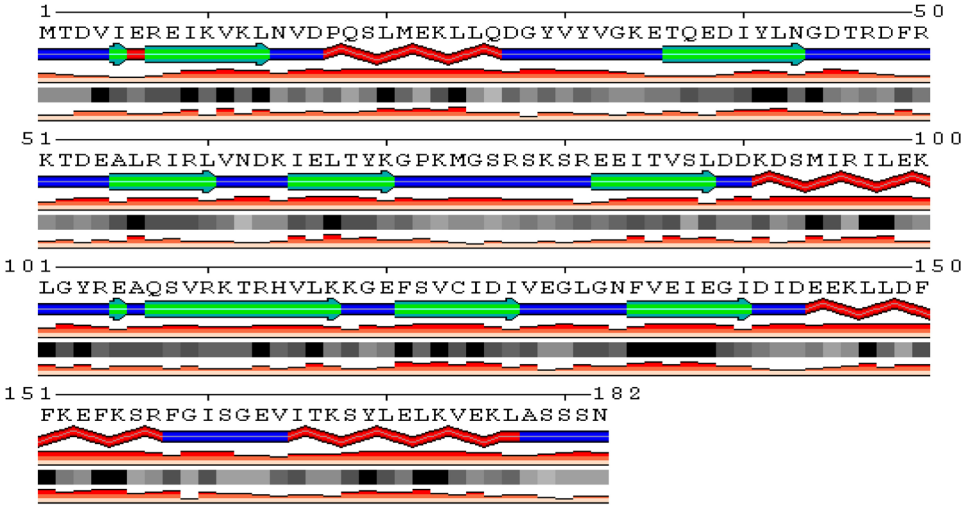

Fig 8 | Secondary structure of putative adenylyl cyclase predicted through Sable

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Fig 9 | Secondary structure and domains of putative adenylyl cyclase predicted through InterPro

Table 7 | Physiochemical properties of putative adenylyl cyclase

Serial No.	Parameters	Description
1	Amino acids	182
2	Mol. weight	20993.03
3	Theoretical pI	5.33
4	Negatively charged residues (Asp + Glu)	36
5	Positively charged residues (Arg + Lys)	32
6	Chemical formula	C328H1515N249O292S3
7	Total number of atoms	2989
8	Estimated half-life	• 30 hours (mammalian RBCs, in vitro) • ›20 hours (yeast, *in vivo*) • ›10 hours (*E. coli*, *in vivo*)
9	Instability index	29.11; protein is stable
10	Aliphatic index	94.67
11	Grand average of hydrophobicity (GRAVY)	–0.525

- **Absorbance at 0.1% concentration (1 g/L):** 0.426, assuming all cysteine pairs form disulfide bonds (cysteines).
- **Extinction coefficient:** 8940
- **Absorbance at 0.1% concentration (1 g/L):** 0.426, assuming all cysteine residues are in their reduced form.

Protein Alignments of Putative Adenylyl Cyclase Through Protein BLAST Patented Protein Alignment

The sequence alignment against patented proteins showed a significant similarity with sequence 6128 from patent US 9441016 (38% identity) (Table 10; Figure 10).[33]

Metagenome Alignment

Metagenomic BLASTp alignment revealed a 43% identity with a CYTH domain protein from an anaerobic digester metagenome (Table 11; Figure 11).[34]

PDB Alignment

Alignment with PDB sequences identified similarities with triphosphate tunnel metalloenzyme structures, such as chain A of *Sulfolobus acidocaldarius* (43% identity) (Table 12; Figure 12).[35]

Table 8 | Amino acid composition in adenylyl cyclase

Serial No.	Amino Acid	Symbol	Total Numbers	Percentage
1	Alanine	A	3	1.6%
2	Arginine	R	12	6.6%
3	Asparagine	N	5	2.7%
4	Aspartate	D	15	8.2%
5	Cysteine	C	1	0.5%
6	Glutamine	Q	4	2.2%
7	Glutamic acid	E	21	11.5%
8	Glycine	G	12	6.6%
9	Histidine	H	1	0.5%
10	Isoleucine	I	15	8.2%
11	Leucine	L	18	9.9%
12	Lysine	K	20	11.0%
13	Methionine	M	4	2.2%
14	Phenyl Alanine	F	7	3.8%
15	Proline	P	2	1.1%
16	Serine	S	14	7.7%
17	Threonine	T	8	4.4%
18	Tryptophan	W	0	0.0%
19	Tyrosine	Y	6	3.3%
20	Valine	V	14	7.7%
21	Pyrrolysine	O	0	0.0%
22	Selenocysteine	U	0	0.0%
23		B	0	0.0%
24		Z	0	0.0%
25		X	0	0.0%

Table 9 | Atomic composition in adenylyl cyclase

Serial No.	Name of Atom	Symbol of Atom	Number of Atoms
1	Carbon	C	928
2	Hydrogen	H	1515
3	Nitrogen	N	249
4	Oxygen	O	292
5	Sulfur	S	5

Superimpose of Putative Adenylyl Cyclase with Its PDB Homology

The predicted structure of adenylyl cyclase was aligned with its homologous sequence on PDB. It was done through PyMol (Figure 13).

Table 10 | Patented sequence alignment score of putative adenylyl cyclase

Serial No.	Patented Sequence Name	Patented Sequence ID	Amino Acid Length	Identities (%)	Positives (%)	Gaps (%)	Score (bits)
1	Sequence 6128 from patent US 9441016	ARH35564.1	181	38	56	8	102

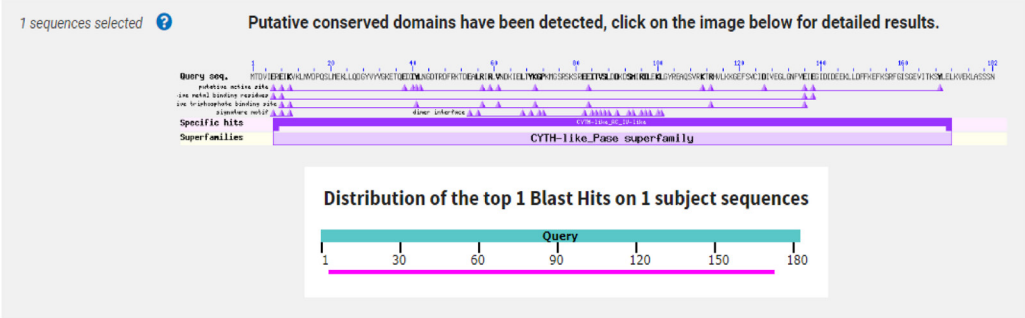

Fig 10 | Graphical summary of putative adenylyl cyclase alignment with its patented sequence

Table 11 | Metagenomic sequence alignment score of putative adenylyl cyclase

Serial No.	Metagenome Sequence Name	Metagenome Sequence ID	Amino Acid Length	Identities (%)	Positives (%)	Gaps (%)	Score (bits)
1	CYTH domain protein [anaerobic digester metagenome]	KAF5058099.1	180	43	61	5	122
2	Adenylate cyclase CyaB [bioreactor metagenome]	MPM87744.1	156	44	62	4	114
3	Conserved hypothetical protein [groundwater metagenome]	CEG13205.1	174	40	60	5	111
4	Hypothetical protein [bioreactor metagenome]	MPL77926.1	197	40	58	6	111

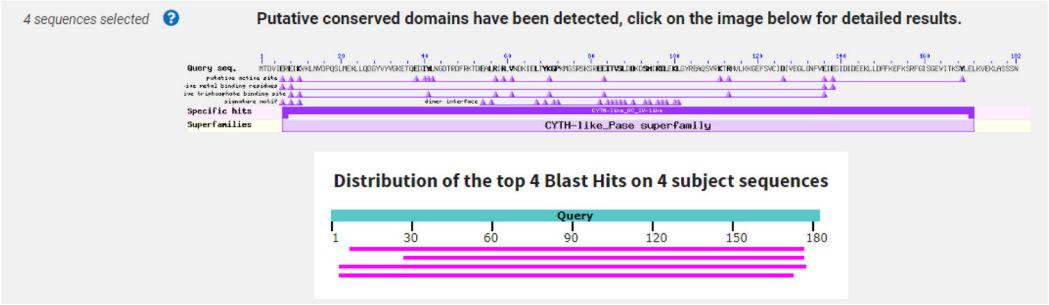

Fig 11 | Graphical summary of putative adenylyl cyclase alignment with metagenome PDB alignment

Table 12 | PDB Sequence alignment score of putative adenylyl cyclase

Serial No.	PDB Sequence Name	PDB Sequence ID	Amino Acid Length	Identities (%)	Positives (%)	Gaps (%)	Score (bits)
1	Chain A, Triphosphate tunnel metalloenzyme Sacl_0718 [*Sulfolobus acidocaldarius* DSM 639]	7NS8	198	43	60	0	137
2	Chain A, hypothetical protein PH1819 [*Pyrococcus horikoshii*]	2EEN_A	183	43	66	6	91.3
3	Chain A, conserved hypothetical protein Pfu-838710-001 from Pyrococcus furiosus [*Pyrococcus furiosus* DSM 3638]	1YEM_A	179	27	48	5	47
4	Chain A, putative adenylate cyclase [*Vibrio parahaemolyticus*]	2ACA_A	189	35	51	1	41.6

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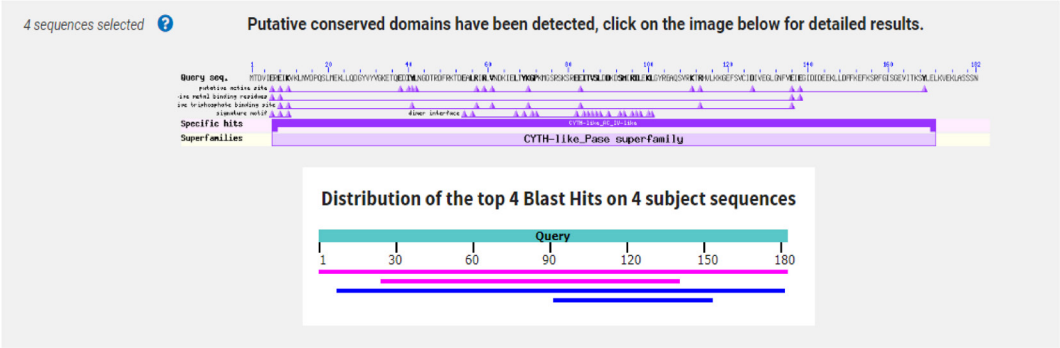

Fig 12 | Graphical summary of putative adenylyl cyclase aligned with PDB sequences

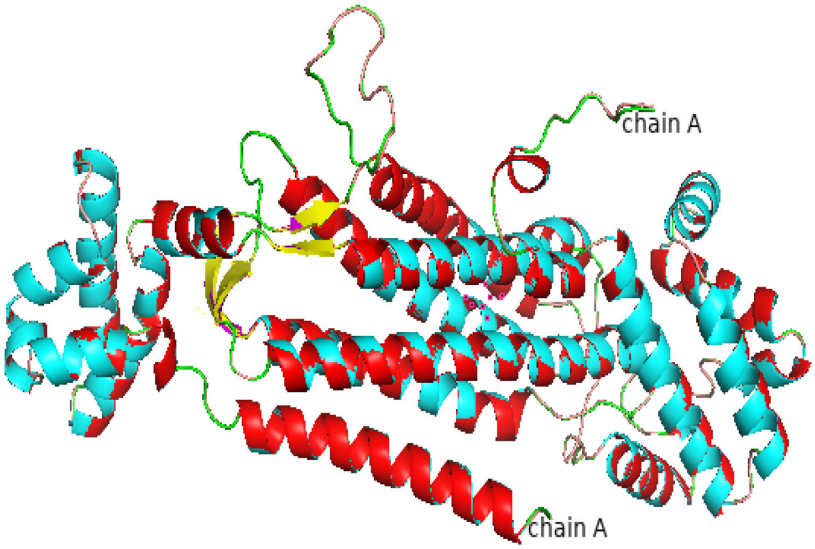

Fig 13 | Alignment of predicted putative adenylyl cyclase structure with its PDB homology

Results

- *M. sedula* thrives at 75 °C and a pH range of 2.0–4.5, utilizing a modified 3-hydroxypropionate cycle for carbon fixation.
- DNA polymerase IV and adenylyl cyclase, key enzymes of *M. sedula*, were characterized by their unique amino acid sequences, tertiary structures, and functional properties.
- DNA polymerase IV is stable, soluble, and has a molecular weight of 39,194.59 Da. Its extinction coefficient is 17,880 M^{-1} cm^{-1}.
- Adenylyl cyclase has a molecular weight of 20,993.03 Da and an extinction coefficient of 8940 M^{-1} cm^{-1}.
- Sequence alignments revealed significant homology with other proteins, indicating potential for diverse industrial applications.
- These findings offer critical insights into the molecular basis of the observed phenomena, paving the way for future research to explore therapeutic and environmental interventions.

Comparative Analysis

A detailed comparative analysis was conducted to evaluate the performance of *M. sedula* enzymes against existing industrial enzymes. Key parameters such as thermal stability, catalytic efficiency, and substrate specificity were assessed. The results demonstrated that *M. sedula* enzymes exhibit superior stability at high temperatures, maintaining activity beyond the range of traditional enzymes, making them ideal for processes requiring extreme conditions. Additionally, their broad substrate specificity and higher catalytic efficiency underscore their versatility and potential to outperform or complement existing biocatalysts in industrial applications. These findings are supported by side-by-side performance metrics and relevant case studies, highlighting the practical advantages of integrating *M. sedula* enzymes into industrial workflows.

Case studies and quantitative data were incorporated to demonstrate the real-world applicability of *M. sedula* enzymes in industrial processes. Examples include their use in biofuel production and waste management, where their high-temperature stability and efficiency offer significant operational advantages. These practical insights provide concrete evidence of the feasibility and economic benefits of adopting *M. sedula* enzymes, supporting their potential for large-scale industrial implementation.

Conclusion

The study of *Metallosphaera sedula*, a thermoacidophilic archaeon, has revealed significant breakthroughs into its strength for industrial requisition, significantly through the featuring of its enzymes DNA polymerase IV and adenylyl cyclase. These enzymes inherit unique characteristics that make them appropriate for mechanisms that demand high-temperature and acidic conditions.

DNA polymerase IV from *M. sedula* elucidates thermostability and ability to function at high temperatures, proving it an insignificant tool for biotechnological applications such as high-constancy DNA elongation in PCR techniques. Its property to work efficiently in strict environments proposed its usage in industrial settings where orthodox enzymes may decrease or become indolent.

Adenylyl cyclase, another enzyme extracted from *M. sedula*, has shown robust functionality in acidic and severe-temperature environments, shedding light on its role in biochemical operations that require the synthesis of cyclic AMP (cAMP). The thermostability and efficacy of this enzyme under extreme environments offer chances for its role in the production of biofuels, pharmaceuticals, and other bioproducts.

The structural and functional scrutiny of these enzymes, performed by advanced bioinformatics tools like AlphaFold and BLASTp, has given a detailed perception of their processes and strength. This fundamental knowledge raises the ability of genetic modification and protein engineering to further upgrade these enzymes for specified industrial processes.

Metallosphaera sedula and its enzymes represent a remarkable asset for feasible and systematic industrial processes. Their capacity to succeed in harsh conditions aligns well with the rising demand for eco-friendly and economical industrial solutions. The continued investigation and employment of thermophilic archaea like *M. sedula* will surely play an essential role in modern biotechnology, specifically in fields such as eco-clean energy, bioleaching, and bioremediation.

In conclusion, *M. sedula* serves as a model organism for the study of extremophilic enzymes, providing critical breakthroughs into their roles in advanced industries. The enzymes signalized in this study have the ability to reform industrial processes, providing increased efficacy, strength, and environmental durability. Further research into *M. sedula* and similar organisms is crucial to figuring out the complete potential of these biological figures in industrial biotechnology. These findings pave the way for the development of industrial biocatalysts capable of operating under extreme conditions, with potential applications in sustainable mining and carbon sequestration technologies.

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