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

Ambreen Ilyas¹, Hina Javed¹ and Khadija Batool²

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 *M. 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 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

M. 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 that grow at extreme temperatures up to 110°C 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. They play a crucial role in directing the synthesis of commercially valuable products, specifically for the food, beverage, and chemical industries.

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, *M. sedula* strikes as a laboratory organism for analyzing biomining and carbon fixation in severe environments.³ This thermoproteota demonstrates exceptional adaptability, employing 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 plays 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 studies have provided detailed perceptions into the composition and function of the enzymes, summing their potential for biotechnological roles, such as CO₂ fixation and biofuel production.^{8,9}

Furthermore, the hardness 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. Therefore, 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 hereditary material possesses any form of thermostable DNA polymerases.¹³

According to an estimate of the complete human genome, there are almost 14 DNA-dependent DNA polymerases, 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 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*, which is obtained from *Thermus 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 its 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.
- Physicochemical properties were assessed using Expasy ProtParam.
- Sequence alignments were performed using BLASTp against patented, metagenomic, and Protein Data Bank (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

(Mivlfvdfdyffaqqveeilnpslkgkpvvvcvysgrtkdsgavatsn yearklgikagmpiikakeigkdaiflpmrkevqqvsrrvmniisgyg dkleiasideaylditrrvkdfdeakelarrlkaevlekerlrvtvgigpnkv vakiiadmnkpdglgiypeevkdfnlndiskvpvgkitekierlkag inrlgdvvnksgeivnlvgkskanyllslanntyhdpvesreithrgryvtl pentrdlnrlpslksieeayskvdgipmeiyvvaimeiddimskgks fkgvvsqdralsvaqellnkilesdkrklrrvgvrlgkitkstldflh).

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 following is the secondary structure predicted via SABLE. Green arrows represent the alpha helix, and the red color shows beta sheets (Figure 2).²²



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

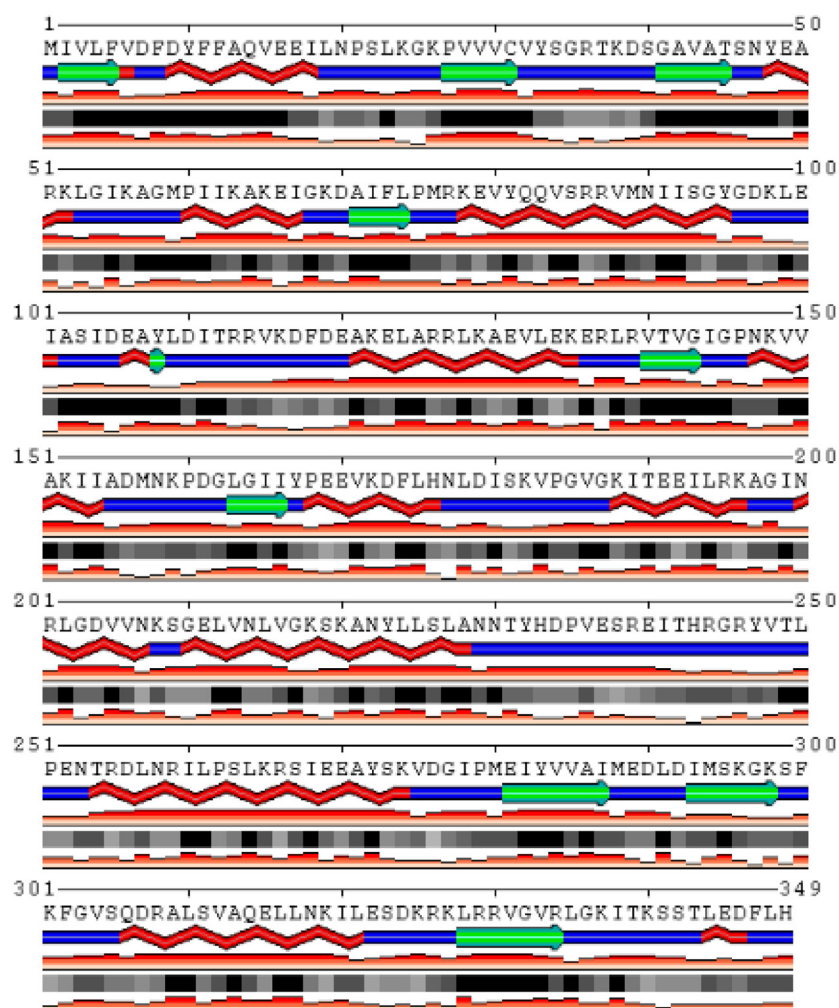


Fig 2 | Secondary structure of DNA polymerase IV predicted through SABLE

Solvent Accessibility

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

Secondary Structure Through InterPro and Domains24

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/Diguanilate cyclase.

Conserved site: Poly_HhH_motif, IMS HHH

Unintegrated: DNA Repair Polymerase UMUC/Trans-ferase family member

Other features: Coil

Residues: Poly_Pol_IV_kappa (Figure 3)

Physiochemical Parameters25

Physiochemical properties of DNA polymerase IV have been predicted through the ExPASy ProtParam tool (Tables 1–3).

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 relevant sequence of DNA polymerase IV was aligned with patented sequences through BLASTp (Figure 4 and Table 4).

Metagenomic Alignment

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

PDB Alignment

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

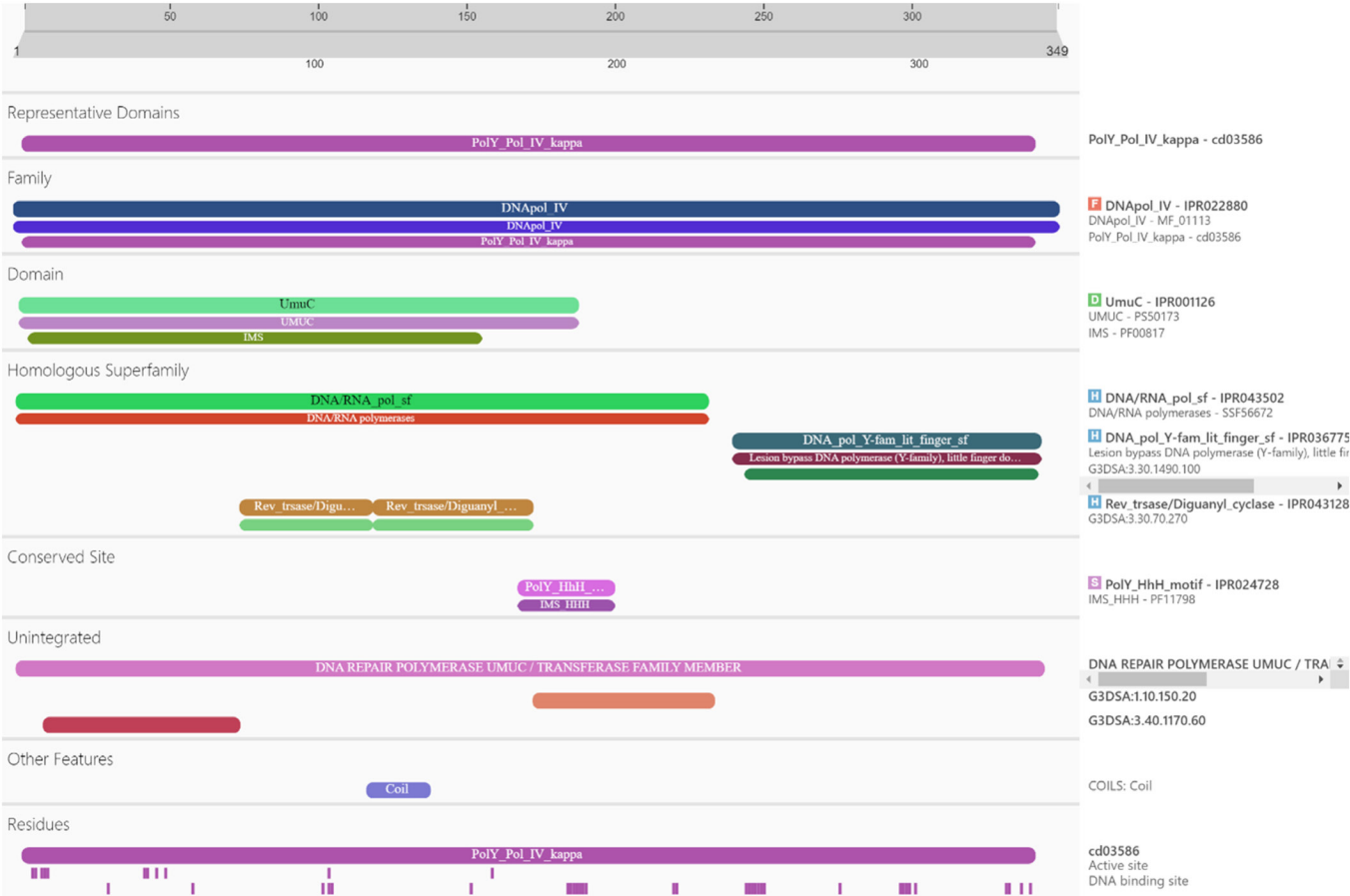


Fig 3 | Secondary structure and domains of DNA polymerase IV predicted through InterPro

Table 1 Physiochemical properties of DNA polymerase IV		
Serial No.	Parameters	Description
1	Amino acids	349
2	Mol. weight	39,194.59
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, <i>in vitro</i>) • >20 hours (yeast, <i>in vivo</i>) • >10 hours (<i>Escherichia coli</i>, <i>in vivo</i>)
9	Instability index	36.57; protein is stable
10	Aliphatic index	104.96
11	Grand average of hydropathy (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	Phenyl Alanine	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

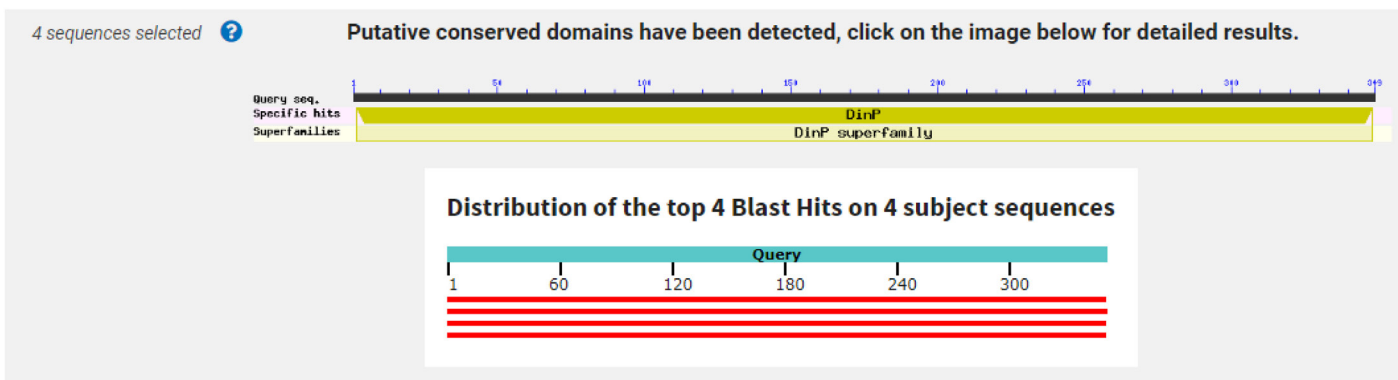


Fig 4 | Patented sequence alignment of DNA polymerase IV

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 US11634741	WON57241.1	351	58	78	0	417
2	Sequence 117 from patent US11634741	WON57016.1	352	57	77	0	397
3	Sequence 17 from patent US9850471	AVD24932.1	352	57	77	0	394
4	Sequence 8 from patent US7745188	ADR91353.1	350	62	78	0	434

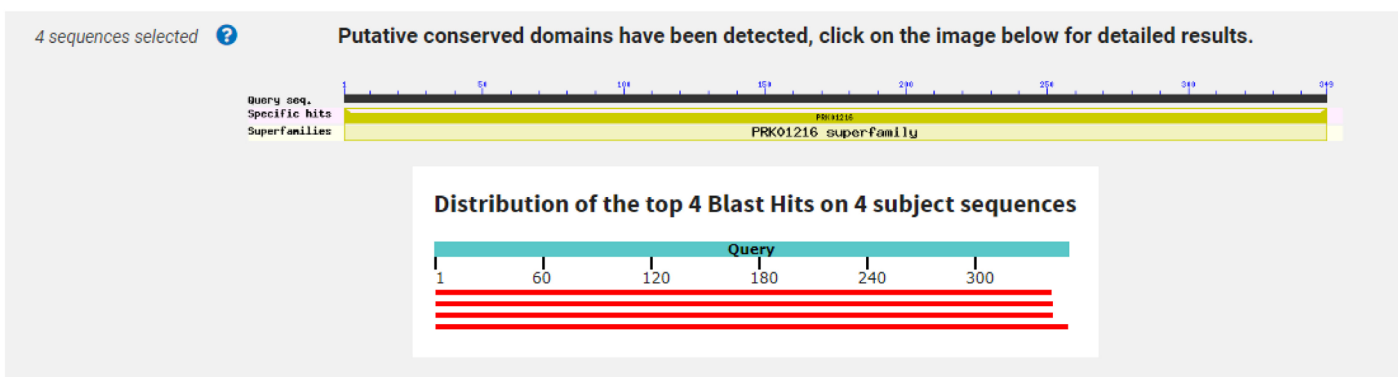


Fig 5 | Metagenome sequence alignment of DNA polymerase IV

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

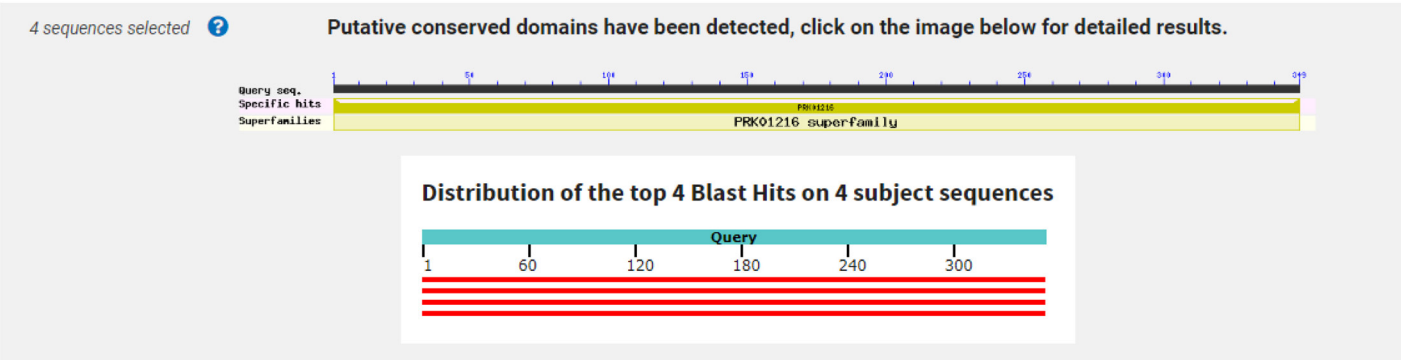


Fig 6 | PDB sequence alignment of DNA polymerase IV

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 [<i>Sulfolobus acidocaldarius</i>]	3BQO_A	354	59	78	1	423
2	Chain A, DBH protein [<i>Saccharolobus solfataricus</i>]	1K1Q_A	354	59	78	1	418
3	Chain A, DNA polymerase IV [<i>S. acidocaldarius</i> DSM 639]	4F4Y_A	362	59	78	1	418
4	Chain A, DNA polymerase IV [<i>S. solfataricus</i> P2]	4F4Z_A	361	60	78	0	411

Putative Adenylyl Cyclase

The thermostable characteristics of putative adenylyl cyclase are based on its ability to function and remain stable even at high temperatures, which is 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 functions, ranging 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 can contribute to robust and innovative advancements in molecular biology and biotechnology.³²

Up to today, nine isoforms of this enzyme have been cloned related to mammals, having the 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 (MTD VIEREIKVKLVNDPQSLMEKLLQDGYVYVGKETQEDI YLNGDTRDFRKTDEALRIRLVNDKIELTYKG PKMGSRSKSREEITVSLDDKDSMIRILEKLG Y REAQSVRKTRHVLKKG EFSVCIDIVEGL

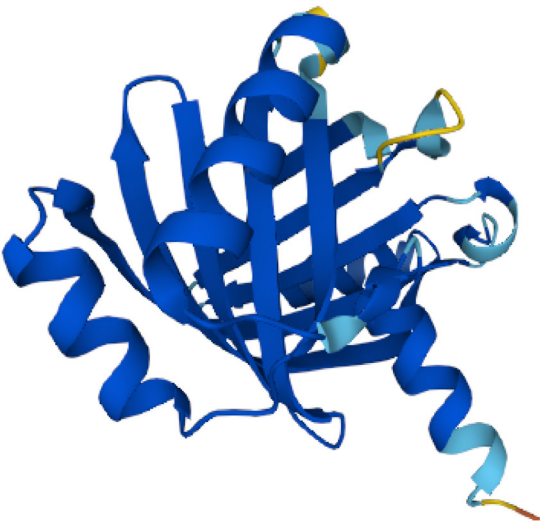


Fig 7 | Tertiary structure of putative adenylyl cyclase predicted through AlphaFold

GNFVEIEGIDIDEKLLDFFKEFKSRFGIS GEVITKSYLELKVEKLASSN) was obtained through predictive modeling software; Uniprot.²⁷

Tertiary Structure Predicted Through AlphaFold
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 Predicted Through SABLE
Secondary structure analysis using SABLE revealed regions corresponding to beta sheets (red) and alpha helices (green), consistent with its functional domains (Figure 8).²⁹

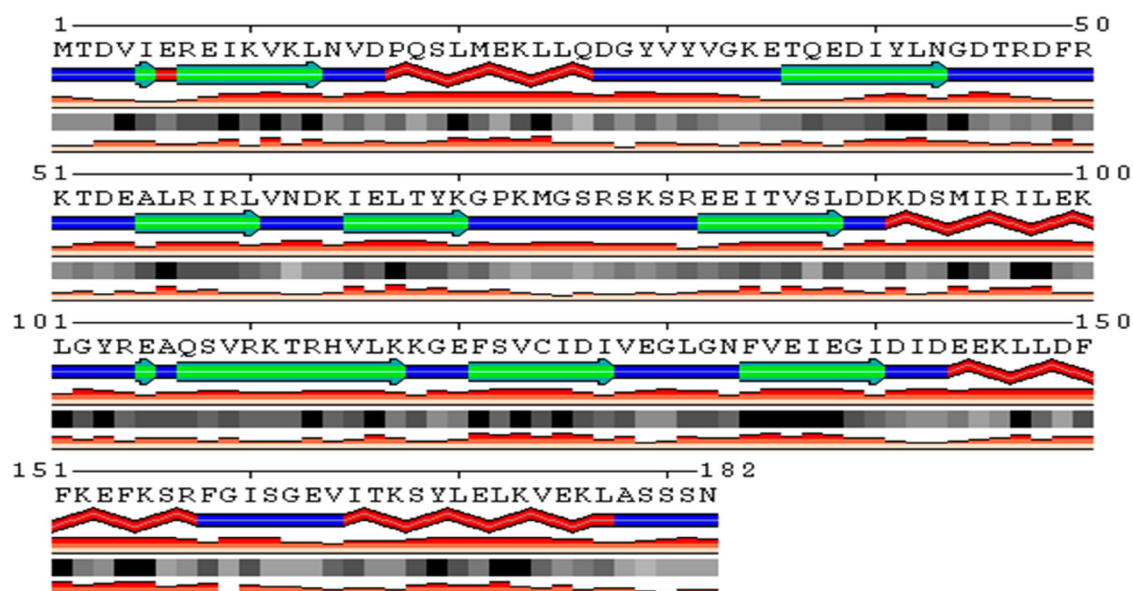


Fig 8 | Secondary structure of putative adenyl cyclase predicted through SABLE

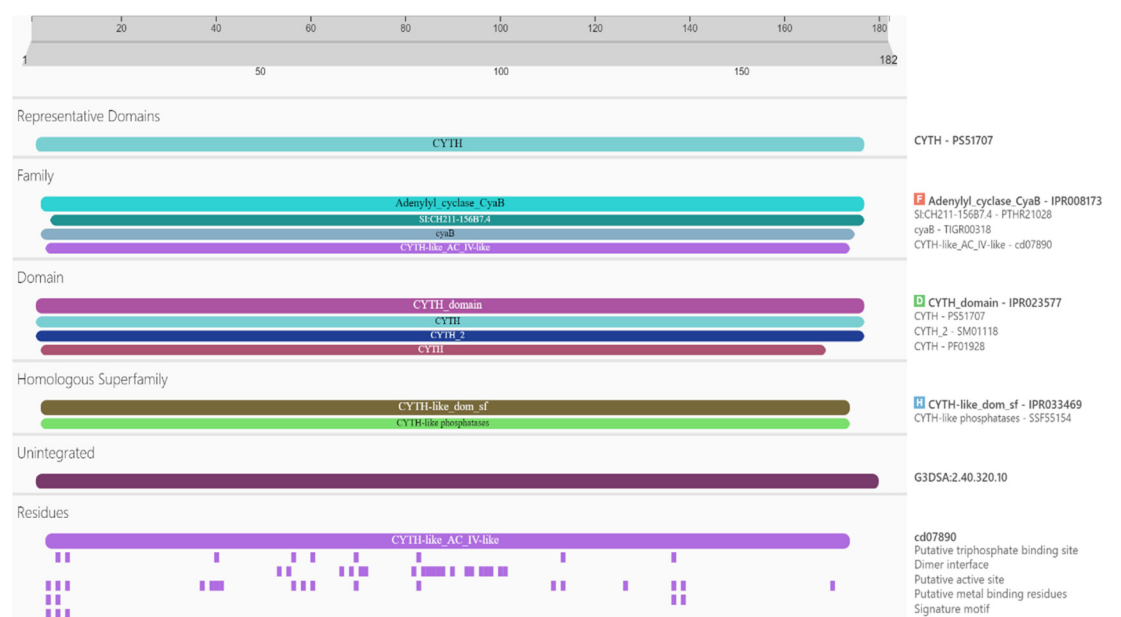


Fig 9 | Secondary structure and domains of putative adenyl cyclase predicted through InterPro

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 adenyl cyclase_CyaB family.³¹

Representative domains: CYTH

Family: Adenyl 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 ProtParam tool, revealing a molecular weight of 20,993.03 and a theoretical pI of 5.33 (Tables 7–9).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.

Table 7 | Physiochemical properties of putative adenylyl cyclase

Serial No.	Parameters	Description
1	Amino acids	182
2	Mol. weight	20,993.03
3	Theoretical pI	5.33
4	Negatively charged residues (Asp+Glu)	36
5	Positively charged residues (Arg+Lys)	32
6	Chemical formula	C328H515N249O292S3
7	Total number of atoms	2989
8	Estimated half-life	• 30 hours (mammalian RBCs, <i>in vitro</i>) • >20 hours (yeast, <i>in vivo</i>) • >10 hours (<i>E. coli</i>, <i>in vivo</i>)
9	Instability index	29.11; protein is stable
10	Aliphatic index	94.67
11	Grand average of hydropathy (GRAVY)	-0.525

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

- **Extinction coefficient:** 8940
- **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 US9441016 (38% identity) (Figure 10 and Table 10).³³

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

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

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).

- 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.

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

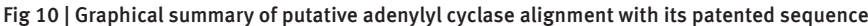


Table 10 | Patented sequence alignment score of putative adenylyl cyclase

Serial No.	Patented Sequence Name	Patented Sequence ID	Amino Acid Length	Identities (%)	Positives (%)	Gaps (%)	Score (bits)
1	Sequence 6128 from patent US9441016	ARH35564.1	181	38	56	8	102



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

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

This study provides detailed insights into the industrial potential of *M. sedula*, particularly through the

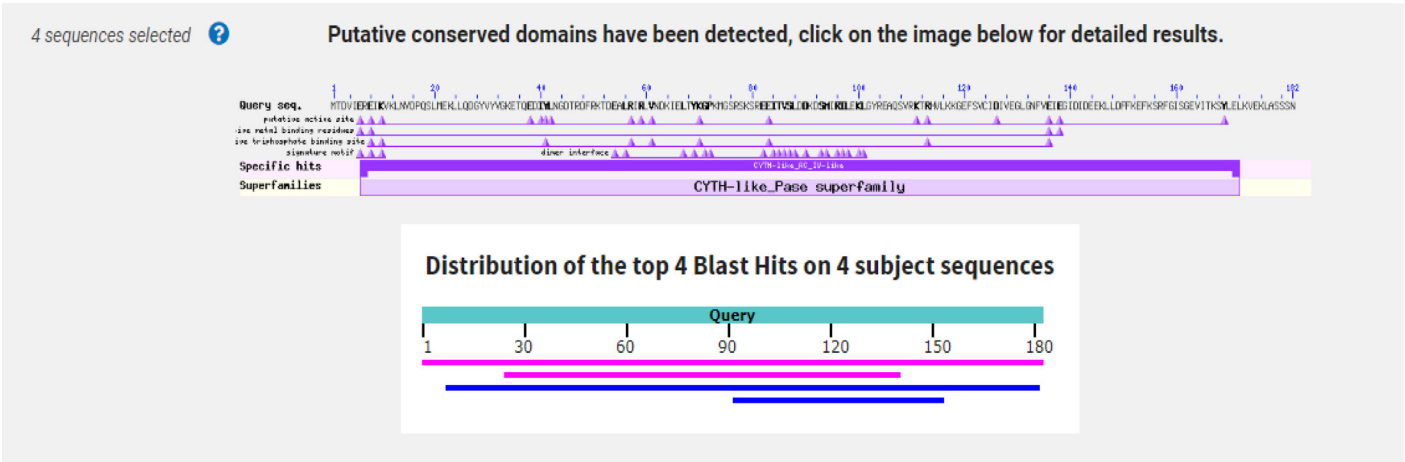


Fig 12 | Graphical summary of putative adenylyl cyclase aligned with PDB sequences

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 SacI_0718 [S. 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 [P. 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

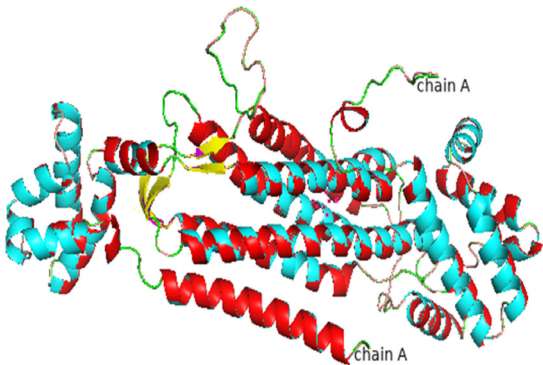


Fig 13 | Alignment of predicted putative adenylyl cyclase structure with its PDB homology

characterization of its enzymes, DNA polymerase IV, and adenylyl cyclase. DNA polymerase IV exhibits high thermostability and efficient catalytic activity, making it a promising candidate for high-fidelity DNA amplification in PCR and other biotechnological applications. Adenylyl cyclase, with its robust activity under acidic and high-temperature conditions, has potential applications in cyclic AMP synthesis, biofuel production, and pharmaceutical processes. The structural and functional analyses, supported by bioinformatics tools like AlphaFold and BLASTp, have provided a comprehensive understanding of these enzymes' properties. This knowledge can aid in genetic modifications and

protein engineering to enhance their efficiency for specific industrial applications. Given their ability to function under extreme conditions, *M. sedula* enzymes align with the growing need for resilient and sustainable biocatalysts in industrial processes.

Future research should focus on optimizing these enzymes for large-scale applications, improving their stability and activity under diverse environmental conditions. The findings of this study underscore the potential of *M. sedula* as a valuable resource in industrial biotechnology, particularly in sustainable mining, bioleaching, and carbon sequestration technologies.

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