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AlphaFold modeling and computational analysis of a PHA synthase from *Actinophytocola algeriensis*

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Abstract:

Environmental challenges related to plastic waste underscore the urgent need for innovative solutions. Polyhydroxyalkanoates (PHA) have emerged as sustainable alternatives to conventional plastics, particularly in packaging, due to their biodegradability and biocompatibility. However, the cost of PHA production and certain physical limitations compared to synthetic polymers remain significant barriers to widespread adoption. Within this context, *Actinophytocola algeriensis*, an Actinobacteria species isolated from the Sahara desert in Algeria, holds promise for its biotechnological potential and bioactive molecules. Despite this, our understanding of its enzyme profile, notably the PHA synthase (EC 2.3.1.304), the key enzyme in PHA biosynthesis, remains limited. In this study, the 3D structure of PHA synthase was modeled utilizing the artificial intelligence program AlphaFold, followed by the structural refinement and validation. In addition, physicochemical properties and functional characterization were conducted using various bioinformatics tools. This research signifies a substantial advancement in comprehending the molecular mechanisms underlying PHA biosynthesis in *A. algeriensis*, thereby fostering the development of innovative biotechnological applications for sustainable biopolymer production.

Keywords : Polyhydroxyalkanoates, *Actinophytocola algeriensis*, PHA synthase, bioinformatics tools.

• Introduction

Polyhydroxyalkanoates (PHA) are aliphatic polyesters synthesized and accumulated by various microorganisms under stress conditions, marked by an excess of carbon substrate and a scarcity of other essential nutrients like nitrogen, sulfur, phosphorus, or oxygen. These biopolymers show great promise with diverse applications in biotechnology, particularly in producing environmentally friendly plastics. Their biodegradability, biocompatibility, and adaptability have garnered substantial interest,

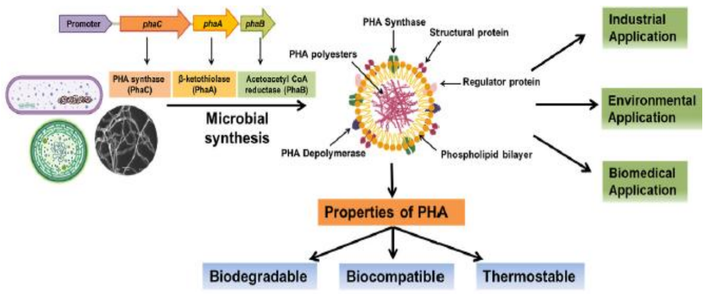

Fig. 1. Polyhydroxyalkanoates, the bioplastics of microbial origin: Properties, biochemical synthesis, and their applications (Behera *et al.*, 2022).

serving not only as an eco-conscious alternative to conventional petroleum-based plastics but also for specialized uses, including agricultural and biomedical devices (**Figure 1**). The key enzyme for PHA biosynthesis is PHA synthase (Polyhydroxyalkanoate synthase, EC 2.3.1.304), or PhaC, catalyzing the polymerization of hydroxyalkanoates (HAs) from HA-CoA and releasing CoA (coenzyme A) during the reaction. Furthermore, Newly identified PhaCs fall outside the established four classes. Over the past two decades, the actinobacteria phylum, including genera like *Nocardia*, *Rhodococcus*, *Leifsonia, Microbacterium, Paenarthrobacter*, *Arthrobacter, Kineosphaera*, and *Streptomyces* have been identified as producers of PHA and PHA synthase. However, no studies have been conducted on this topic regarding the genus *Actinophytocola. Actinophytocola algeriensis*, isolated from Algeria's Sahara desert soil, exhibited biotechnological promise and contained bioactive molecules such as PHA synthase. Thus, more investigation is necessary to bridge this knowledge gap and uncover the potential uses of this enzyme. Nonetheless, the experimental process of expressing and purifying proteins can present challenges, demanding considerable time and effort. Therefore, The approach of *in silico* methods offers a viable solution to these challenges, allowing researchers to gain insights into the functional and structural properties of proteins. In this study, PHA synthase from *A. algeriensis* was subjected to *in silico* analysis, revealing its physicochemical, functional, and secondary structural properties, and undergoing protein homology modeling.

• Materials and methods

Computational methods	Computational tools
1. Amino acid sequence retrieval	Uniprot (ID: A0A221VZV6)
2. Physicochemical properties prediction	ExPASy ProtParam and SOSUI
3. Secondary structure characterization	SOPMA
4. AlphaFold modeling and evaluation of the tertiary structure	AlphaFold, ModRefiner, PyMOL, and PROCHECK
5. Funtional analyses	SOSUI, COFACTOR, and CDD

Table1. Parameters computed for the PHA synthase from *A. algeriensis* using Expasy's Protparam.

Property	Value
Number of amino acids residues (AA)	350
Molecular weight (Da)	38319. 24
Theoretical pI	7.10
Total number of negatively charged residues (Asp + Glu)	39
Total number of positively charged residues (Arg + Lys)	39
Extinction coefficient (EC)	35535
Instability index (II)	35.35 (Stable)
Aliphatic index (AI)	100.86
Grand average of hydropathicity (GRAVY)	0. 071

Table2. Secondary structure prediction of the the PHA synthase from *A. algeriensis* by SOPMA SERVER

Property	Value
Alpha helix	43.14 %
310 helix	0.00 %
Pi helix	0.00 %
Beta bridge	0.00 %
Extended strand	14.29 %
Beta turn	5.14 %
Bend region	0.00 %
Random coil	37.43 %
Ambiguous state	0.00 %
Other states	0.00 %

Table3. Ramachandran plot analysis of refined PHA synthase model using PROCHECK

Favored region (%)	Additional allowed region (%)	Generously allowed region (%)	Disallowed region (%)
93.0	6.4	0.7	0.0

• Results and discussions

Table4. Transmembrane regions identified in the PHA synthase using SOSUI server

No.	N terminal	Transmembrane sequence	C terminal	Type	Length
1.	130	GGAPVHVVAWCLGGILSLLTHAD	152	Primary	23
2.	159	ASIATIAAPIDMTAIPLVAPIKP	181	Secondary	23

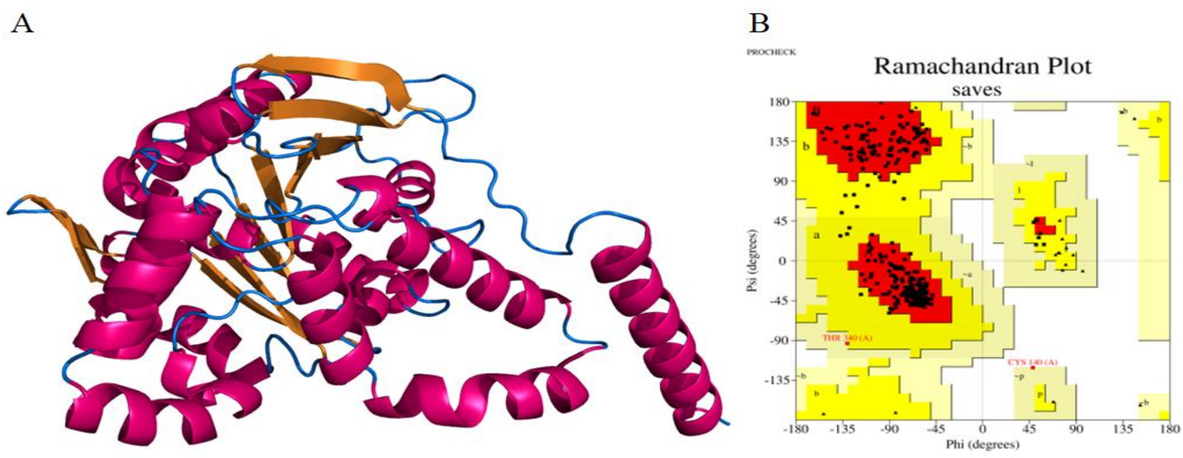

Fig. 2. Tertiary structure prediction and validation for the PHA synthase from *A. algeriensis.*
(A) The protein's tertiary structure was predicted using AlphaFold modeling, refined by ModRefiner, and visualized with PyMOL. The structural features, including α-helix (pink), β-sheet (orange), and loop (marine), were identified.
(B) The predicted and refined structure's Ramachandran plot was validated using the PROCHECK program, classifying residues based on their conformational preferences. Regions with favored (A, B, and L), additional allowed (a, b, l, and p), generously allowed (~a, ~b, ~l, and ~p), and disallowed conformations are highlighted accordingly. Non-glycine and non-proline residues are represented by filled black squares, while glycines (excluding those at the ends of the polypeptide chains) are depicted as filled black triangles.

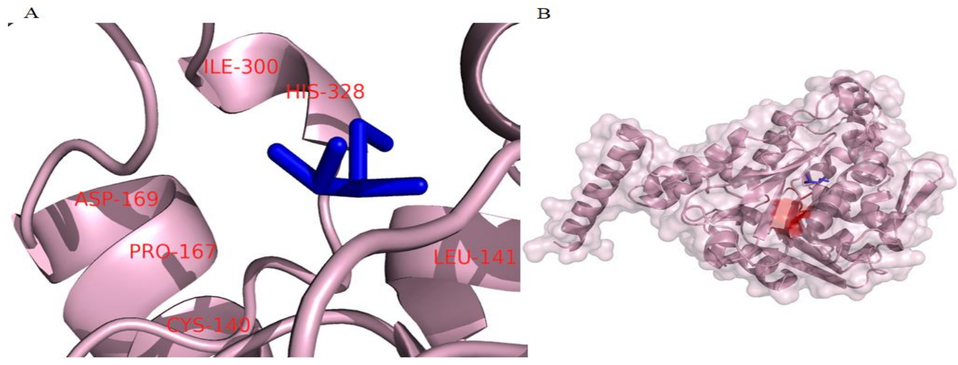

Fig. 3. Ligand binding sites of the *A. algeriensis* PHA synthase predicted by COFACTOR. (A) Ligand binding sites (Cys 140, Leu 141, Pro 167, Asp 169, Ile 300, and His 328).
(B) Surface view transparency of the protein with ligand. Sticks represent the ligand (blue). Ligand binding sites (red) within the protein structure (pink).

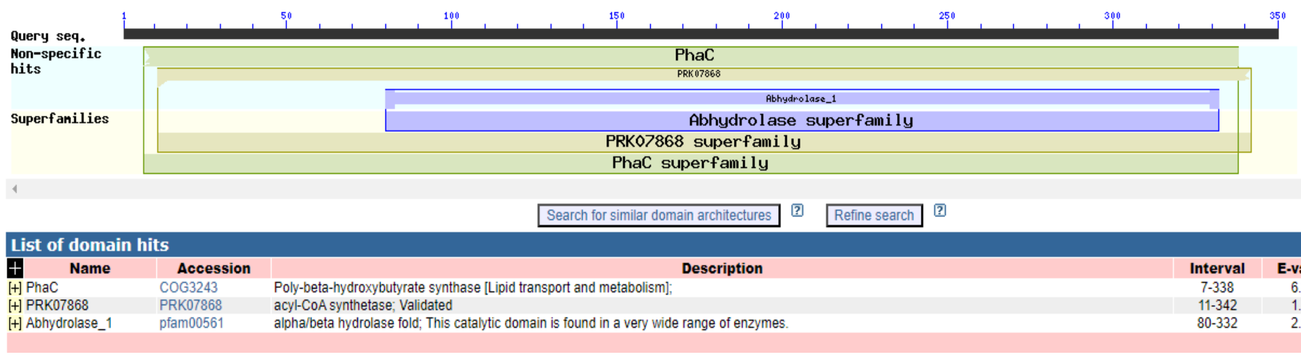

Fig. 4. Analysis of *A. algeriensis* PHA Synthase Domains Using the CDD Program.

• Conclusions

The computational analysis of the PHA synthase from *A. algeriensis* uncovered several noteworthy characteristics; its neutrality, thermostability, hydrophobicity, and membranar localization. The protein's secondary structure prediction consists mostly of alpha helices and random coils, with additional elements of extended strands and beta turns. Structural modeling and functional analyses were conducted, suggesting that this computational approach holds promise for designing PHA synthases with industrial applications. However, additional *in vivo* studies, along with experimental investigations like enzyme kinetics and substrate specificity, are warranted to fully explore its industrial potential.

• References

- Behera, S., Priyadarshanee, M., & Das, S. (2022). Polyhydroxyalkanoates, the bioplastics of microbial origin: Properties, biochemical synthesis, and their applications. *Chemosphere, 294*, 133723. DOI: 10.1016/j.chemosphere.2022.133723

- Bouznada, K., Bouras, N., Schumann, P., Spröer, C., Sabaou, N., & Klenk, H.-P. (2016). *Actinophytocola algeriensis* sp. nov., an actinobacterium isolated from Saharan soil. *International journal of systematic and evolutionary microbiology, 66*(7), 2760-2765. DOI: 10.1099/ijsem.0.001136

- Neoh, S. Z., Chek, M. F., Tan, H. T., Linares-Pastén, J. A., Nandakumar, A., Hakoshima, T., & Sudesh, K. (2022). Polyhydroxyalkanoate synthase (PhaC): The key enzyme for biopolyester synthesis. *Current Research in Biotechnology, 4*, 87-101. DOI: 10.1016/j.crbiot.2022.01.002