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EXPLORING THE EVOLUTIONARY RELATIONSHIPS AND IMPACT OF METACASPASE PROTEINS IN HARMFUL ALGAL BLOOMS IN THE UAE WATERS

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ABSTRACT

Harmful algal blooms (HABs) pose significant threats to aquatic ecosystems, human health, and coastal economies worldwide. The causative agents of HABs are primarily dinoflagellates (*Marglefidiniumpolykrikoides* and *Prorocentrum minimum*), and diatoms (*Chaetoceros tenuissimus*, *Fragilaria radians*, *Phaeodactylumtricornutum*, and *Skeletonemamarinoidi*), which produce various toxins that exacerbate the harmful effects. Metacaspase, a toxin-producing protein, has emerged as a critical component in the pathogenesis of HABs. This study aims to investigate the evolutionary relationships among diatoms and dinoflagellates based on the metacaspase protein. The analysis based on sequence clustering, indicates that the dinoflagellates and diatoms share common ancestors where *Marglefidiniumpolykrikoides* and *Prorocentrum minimum* are evolutionarily closer to diatom *Skeletonemamarinoidi*. This study prepares the base to further explore their involvement in HABs, elucidate the production of metacaspase by specific diatom and dinoflagellate species, and investigate the role of metacaspase in the toxicity of HABs.

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INTRODUCTION

The environmental impact of harmful algal blooms (HABs) has been recognized as a major issue worldwide, including in the UAE[1]. Warm water temperatures and an abundance of sunlight foster the rapid growth and bloom formation of HAB species. Moreover, the prolonged survival of HABs is facilitated by the insufficient cleansing and circulation of water in particular coastal locations, which in turn increases the HABs' influence on the marine environment. The proliferation of harmful algal species leads to the depletion of oxygen in the water column, resulting in hypoxic or anoxic conditions. These conditions can result in mass mortality of fish, crustaceans, and other marine organisms, thereby disrupting the local food web and biodiversity[2]. In addition, the release of hazardous toxins by HAB species can result in the deposition of toxins in marine organisms, posing a threat to aquatic life and human health through the consumption of contaminated seafood[3]. The coastal areas of the United Arab Emirates are essential for commercial activities such as fishing, aquaculture, and tourism. HAB outbreaks have negative effects on these industries. Furthermore, HABs can discourage visitors from visiting affected coastal areas, thereby influencing the tourism industry's revenue [4]. Although HABs occur globally, including in the United States and Europe, the species of harmful algae implicated in HABs can differ by region. The UAE experiences blooms dominated by dinoflagellates (*Marglefidiniumpolykrikoides* and *Prorocentrum minimum*), and diatoms (*Chaetoceros tenuissimus*,

Fragilaria radians, *Phaeodactylumtricornutum*, and *Skeletonemamarinoidi*), whereas other regions may have different dominant species, such as *Karenia brevis* in the Gulf of Mexico or *Alexandrium* spp. in the North Atlantic. Metacaspase, a protein associated with programmed cell death, has emerged as a major contributor to HABs' toxicity[5]. Metacaspases are proteases involved in numerous cellular processes, such as apoptosis-like cell death and the regulation of immune responses. Recent studies suggest that metacaspase plays a crucial role in the pathogenesis and toxicity of HABs; however, its specific functions and evolutionary relationships across diatoms and dinoflagellates are inadequately understood. Understanding the evolutionary relationships between diatoms and dinoflagellates, their participation in harmful algal blooms (HABs), and the function of metacaspase in the toxicity of these blooms is essential for the development of effective monitoring and mitigation strategies[6] This study aims to elucidate the evolutionary relationship among diatom and dinoflagellate species based on HAB-linked metacaspase protein.

MATERIALS AND METHODS

Metacaspase protein sequences of *Chaetoceros tenuissimus*, *Fragilaria radians*, *Phaeodactylumtricornutum*, *Skeletonemamarinoidi*, *Marglefidiniumpolykrikoides*, and *Prorocentrum minimum* were obtained from NCBI. The key used is (metacaspase [Protein Name])

AND dinoflagellate [organism] (diatoms[organism] for diatoms search). The accession numbers retrieved are as follows, Marglefidiniumpolykrikoides: AVD53614.1; Proocentrum minimum: QZB49251.1; Chaetocerotenuissimus: GFH57094.1; Phaeodactylumtricornutum CCAP 1055/1: XP_002178108.1; Fragilaria radians: MOB19376.1; Skeletonemamarinoi: ANA05825.1

The sequences were downloaded in FASTA format and stored for further analysis: BLAST (Basic Local Alignment Search Tool) searches were performed to compare the metacaspase protein sequences. Two separate BLAST experiments were conducted. In one of the experiments, the metacaspase protein sequence of Chaetocerotenuissimus is used as the query sequence in a BLAST search against the database of metacaspase protein sequences of rest of the dinoflagellate's downloaded sequences. Similarly, the metacaspase protein sequence of Proocentrum minimum is used as the query sequence in a BLAST search against the metacaspase protein sequences of other diatoms in our study. For the second experiment, the metacaspase protein of both dinoflagellates are used as queries to run BLAST against all of the diatoms in 2 sets. The BLAST analysis was performed using the blastp program with the following parameters: word size of 3, an expected value of 0.05, a hitlist size of 100, a gap cost of 11.1, the BLOSUM62 scoring matrix, and the filter string set to "F". The genetic code used for translation was code 1, and the window size and threshold for the compositional-based statistics were set to 40 and 40, respectively. The Karlin-Altschul statistics for the analysis yielded the following parameters: lambda values of 0.317 and 0.2, k values of 0.135052 and 0.041, and H values of 0.395194 and 0.14, respectively. These parameters provide information about the scoring system used to assess sequence similarity and significance in the BLAST analysis. The alignment parameters included a gap penalty of -11.1 and an end-gap penalty of -5.1. These penalties affect the scoring of alignments and influence the alignment quality and sensitivity. Additionally, the Conserved Domain Database (CDD) parameters were employed, with RPS BLAST enabled, a Blast E-value threshold of 0.003, and the option to find conserved columns and recompute. The distance tree was plotted later-on using default parameters.

Furthermore, the BLAST analysis between Chaetocerotenuissimus and Skeletonemamarinoi also revealed a significant alignment. The total score of 180, a query cover of 77%, and a low E-value of 5e-41 suggest a strong similarity between their metacaspase proteins. The percent identity of 42.24% indicates some sequence divergence, potentially reflecting evolutionary divergence or functional differentiation. The combined results suggest that despite some level of sequence divergence, the metacaspase proteins of Chaetocerotenuissimus show significant similarity to those of Phaeodactylumtricornutum, Fragilaria radians, and Skeletonemamarinoi. These findings indicate potential functional conservation or shared evolutionary ancestry among these diatom species in terms of metacaspase function. Similarly, the BLAST analysis revealed a significant similarity between the metacaspase protein sequences of Proocentrum minimum and Marglefidiniumpolykrikoides. The total score of 103 indicates a strong alignment between the two sequences. The query cover of 60% suggests that a substantial portion of the Proocentrum minimum metacaspase protein sequence aligned with the Marglefidiniumpolykrikoides metacaspase protein sequence. The percent identity of 33.33% indicates that the metacaspase proteins of Proocentrum minimum and Marglefidiniumpolykrikoides have diverged to a certain extent. This suggests potential evolutionary divergence or functional differentiation between the metacaspases in these dinoflagellate species. A percent identity of 33.33% implies that approximately 33.33% of the amino acid residues in the aligned regions are identical between the two sequences. The low e-value of 9e-31 indicates a highly significant match, suggesting a strong likelihood of homology between the metacaspase proteins of both the dinoflagellates. These results suggest a potential relationship and functional similarity between the metacaspase proteins of both the dinoflagellates, despite some level of sequence divergence. The observed sequence similarity could indicate conserved functional domains or structural motifs that are important for the biological functions of metacaspases in these dinoflagellate species. For the comparison between dinoflagellate Marglefidiniumpolykrikoides and the diatoms revealed a significant alignment between their metacaspase proteins.

Table 1. BLASTp analysis results for Dinoflagellates and Diatoms

Query	Organisms	Total score	Percent identity	E-Value	Query cover %
Chaetocerotenuissimus	Phaeodactylumtricornutum	199	30.59	2e-53	91
	Fragilaria radians	170	31.63	1e-51	82
	Skeletonemamarinoi	180	42.24	5e-41	77
Proocentrum minimum	Marglefidiniumpolykrikoides	103	33.33	9e-31	60
Marglefidiniumpolykrikoides	Phaeodactylumtricornutum	33.5	21.55	3e-05	39
	Fragilaria radians	30.8	21.05	2e-04	32
	Skeletonemamarinoi	70.9	29.44	3e-17	55
	Chaetocerotenuissimus	55.1	31.31	2e-12	33
Proocentrum minimum	Phaeodactylumtricornutum	55.1	25.47	4e-12	53
	Fragilaria radians	44.3	22.29	1e-08	54
	Skeletonemamarinoi	85.9	26.39	2e-22	60
	Chaetocerotenuissimus	79.7	31.41	9e-21	52

RESULTS AND DISCUSSION

The results of the BLAST analysis for Chaetocerotenuissimus against Phaeodactylumtricornutum revealed a significant alignment between the metacaspase proteins of these two diatom species (Table 1). The high total score of 199, a query cover of 91%, and a low E-value of 2e-53 indicate a strong similarity between the metacaspase proteins of these diatoms. Although the percent identity was relatively low at 30.59%, it suggests potential evolutionary divergence or functional differentiation between their metacaspases. Similarly, the BLAST analysis between Chaetocerotenuissimus and Fragilaria radians demonstrated a significant alignment between their metacaspase proteins. The total score of 170, a query cover of 82%, and a low E-value of 1e-51 indicate a strong similarity. The percent identity of 31.63% suggests some level of sequence divergence, possibly reflecting evolutionary divergence or functional differentiation between their metacaspases.

The total score of this comparison range between 30 to 70 with the query cover of 32% to 55% and e value around under 3e. The Skeletonemamarinoi shows highest similarity with the Marglefidiniumpolykrikoides. Similarly, the comparison of dinoflagellate Proocentrum minimum with the diatoms revealed strong similarity between the metacaspase protein. The total score ranges between 44.3 to 85.9 with query coverage ranging between 53% to 66%. The combined results suggest that despite some level of sequence divergence, the metacaspase proteins of dinoflagellates show significant similarity to the diatoms. These observations imply potential functional conservation or shared evolutionary ancestry of metacaspases among these dinoflagellate species. To investigate the phylogenetic relationships among the diatoms and dinoflagellates analyzed in this study, a family lineage tree was generated based on the metacaspase protein sequences clustering by BLAST. The tree revealed two main divergent nodes, indicating distinct evolutionary lineages within this protein family. At the first node, the tree diverged

into two branches. One branch led to the diatom *Phaeodactylumtricornutum*, while the other branch led to the diatom *Fragilaria radians*. This branching pattern suggests a common ancestor for these diatoms, followed by subsequent divergence. The second node further divided into two branches. The first branch terminated with the diatom *Chaetocerotenuissimus*, indicating a separate evolutionary trajectory for this species. The second branch further diverged into two additional nodes. The first node led to the diatom *Skeletonemamarinoi*, representing a distinct evolutionary lineage within this group. The second node further diverged into two branches, with one branch leading to *Marglefidiniumpolykrikoides* and the other to *Prorocentrum minimum*. The observed clustering of species within the tree suggests both shared evolutionary history and functional divergence. This branching pattern indicates separate evolutionary paths for these two dinoflagellates. This pattern indicates the common ancestry of the diatoms and dinoflagellates where the diatom *Skeletonemamarinoi* and dinoflagellates *Marglefidiniumpolykrikoides* and *Prorocentrum minimum* are evolutionarily closer than other diatoms. Overall, sequence similarity searches and the family lineage tree provide valuable insights into the functional similarities, evolutionary relationships, and diversification of metacaspase proteins among the diatoms and dinoflagellates studied. These findings contribute to our understanding of the various aspects of harmful algal blooms and their causative agents; However, it is important to note that this analysis is based on sequence data, its clustering and furtherin-depth studies incorporating additional molecular markers and experimental validation are required to enhance our understanding of the evolutionary dynamics and functional implications.

Conflicts of interest: The authors declare that they have no conflict of interests.

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