Vietnam Journal of Biotechnology **23**(4): 543-554, 2025. DOI: 10.15625/vjbt-22699

STRUCTURAL CHARACTERISTICS OF FUCOIDANASES FROM MARINE MICROORGANISMS

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Received: 14.04.2025
Accepted: 03.10.2025

ABSTRACT

Fucoidanase is an enzyme that catalyzes the degradation of fucoidan, a complex sulfated polysaccharide found in brown algae, through the hydrolysis of glycosidic bonds between fucose units. This enzyme plays a crucial role in harnessing and optimizing the biological applications of fucoidan, such as immune support, anti-cancer properties, and anti-inflammatory effects. Marine microorganisms are considered a rich source of fucoidanases due to their adaptation to fucoidan-rich environments. This report focuses on the relationship between the structure of fucoidanases from marine microorganisms and its catalytic function on fucoidan substrates, including the active site with conserved amino acids essential for substrate binding, domain structures that facilitate fucoidan recognition, and the enzyme's selectivity for specific glycosidic linkages. Studies on the tertiary and quaternary structures reveal that the enzyme's spatial configuration not only enables precise fucoidan binding but also ensures stable activity in marine environments. Furthermore, comparative analysis of fucoidanases derived from different marine bacterial strains reveals structural variations that influence their substrate specificity and catalytic efficiency. Notably, the presence of calcium ions (Ca^{2+}) has been shown to play a significant role in stabilizing the enzyme's three-dimensional conformation, maintaining its catalytic integrity, and enhancing its activity under saline conditions commonly found in marine ecosystems. Calcium-binding motifs observed in some fucoidanase structures may also contribute to maintaining structural rigidity, thus improving resistance to denaturation and extending the enzyme's functional lifespan. Recent advancements in protein modeling and molecular docking have contributed to a deeper understanding of how Ca^{2+} interacts with the enzyme and supports substrate recognition. These insights pave the way for future enzyme engineering efforts aimed at improving fucoidanase stability, activity, and industrial applicability in the production of bioactive oligosaccharides.

Keywords: Fucoidan, fucoidanase, marine microorganisms, oligosaccharides, tertiary structure.

INTRODUCTION

Fucoidan, a fucose-containing sulfated polysaccharide primarily found in the cell walls of brown marine algae, has attracted considerable interest due to its wide range of bioactivities, including antioxidant, anticoagulant, anti-tumor, anticancer, anti-thrombotic, anti-inflammatory, and immunomodulatory activities (Ale *et al.*, 2011; Wang *et al.*, 2019; Ustyuzhanina *et al.*, 2014). Currently, fucoidan from brown seaweeds is widely commercialized as a functional supplement supporting cancer therapy, especially in Asian and Western markets. Over the past decade, the number of fucoidan-related scientific publications has increased markedly, reflecting its growing relevance in biomedical research (Zayed and Ulber, 2020). Recently, several studies have moved fucoidan closer to biomedical applications. Fucoidan-rich extracts from *Saccharina latissima* have significantly improved gut microbial diversity in clinical trials and promote bone repair in enzyme-depolymerized forms (Ohmes *et al.*, 2022; Garcia *et al.*, 2025). Moreover, fucoidan from *Laminaria japonica* significantly improves serum lipid profiles in both animal models and hyperlipidemic patients without hepatic or renal toxicity (Li *et al.,* 2008). Its high molecular weight and complex structure, characterized by diverse glycosidic linkages, sulfate groups, and branching patterns, necessitate enzymatic hydrolysis for improved biomedical and biotechnological utilization (Zayed and Ulber, 2020; Zayed *et al.*, 2023). Enzymatic hydrolysis of fucoidan is widely recognized as an advanced and environmentally friendly technology, as it enables precise cleavage of glycosidic bonds without the use of harsh chemical reagents, thereby preserving the bioactivity of the resulting oligosaccharides. Studies have shown that enzymatic methods not only enhance the selectivity and efficiency of fucoidan degradation but also reduce the risk of unwanted structural modifications, making them highly suitable for pharmaceutical and biotechnological applications (Thuan *et al.*, 2020; Zayed and Ulber, 2020). Therefore, the identification and detailed characterization of fucoidan-degrading enzymes hold great significance in generating chemically uniform and well-defined bioactive oligosaccharides with enhanced solubility, which are highly relevant for pharmaceutical applications.

Fucoidanase is a specialized glycoside hydrolase (GH) that catalyzes the degradation of fucoidan by cleaving glycosidic bonds between fucose residues within this substrate (Kusaykin *et al*., 2015). These enzymes are primarily found in marine microorganisms, which have adapted to degrade algal polysaccharides in their natural environments (Arai *et al.*, 2022). According to the specific glycosidic linkages they target, fucoidanases are mainly divided into two types: endo-α(1→3)-fucoidanase (EC 3.2.1.211) and endo-α(1→4)-fucoidanase (EC 3.2.1.212). Based on their conserved sequence motifs and structural similarities among characterized fucoidanases, endo-α(1→4)-fucoidanases belong to glycoside hydrolase family 107 (GH107) in the CAZy database (Vickers *et al.*, 2018), while endo-α(1→3)-fucoidanases are mainly classified into GH168 (Shen *et al.*, 2020). Recent research has also identified a group of endo-α(1→3)-fucoidanases belonging to the newly established GH174 family, highlighting the increasing diversity of fucoidan-degrading enzymes (Lombard *et al.*, 2014).

Although fucoidanases have been identified and investigated since the early 2000s, the structural characteristics remained largely

unknown until 2018, when Vickers *et al.* provided key insights through a seminal study (Vickers *et al.*, 2018). By employing X-ray crystallography and NMR analyses, the research team resolved the structures of three GH107 enzymes–MfFcnA, P5AFcnA, and P19DFcnA–which were derived from bacterial species belonging to the family of Flavobacteriaceae and the genus of Psychromonas. Their analysis demonstrated that these enzymes contain a catalytic domain (D1) adopting a $(\beta/\alpha)_8$-barrel fold, where an aspartate (D) residue and a histidine (H) residue functions as the nucleophile and acid/base catalyst, respectively. In addition, the study identified multiple calcium-binding sites, highlighting their essential role in maintaining structural integrity and facilitating enzymatic function (Vickers *et al.*, 2018). Biochemical characterization using carbohydrate–polyacrylamide gel electrophoresis further revealed that P5AFcnA and P19DFcnA act on fucoidans distinct from those targeted by MfFcnA, demonstrating substrate specificity variations within the GH107 family. Since the study by Vickers *et al.* (2018), numerous fucoidanases have been identified and structurally characterized. Notably, recent work by Mikkelsen *et al.* (2023) determined the structure of the endo-α(1,4)-fucoidanase Mef1 from the marine bacterium *Muricauda eckloniae*. X-ray crystallographic analysis revealed that Mef1 adopts a $(\beta/\alpha)_8$-barrel fold, consistent with previously reported GH107 enzymes, and contains calcium-binding sites essential for structural stability and enzymatic function (Mikkelsen *et al.*, 2023). Structural analyses of these newly discovered fucoidanases consistently confirm a high degree of similarity to the architectures reported by Vickers *et al.* (2018), reinforcing the conserved nature of their catalytic domains and calcium-binding sites. These structural properties—including the active site architecture, conserved catalytic residues, and domain organization—are key determinants of enzymatic specificity and efficiency (Vickers *et al.*, 2018). Interestingly, recent studies reveal that truncating the C-terminal region of fucoidanases not only preserves enzymatic activity but also enhances substrate accessibility, significantly boosting catalytic efficiency. Structural and biochemical analyses suggest that while the C-terminal domain contributes to enzyme stability, its removal alleviates steric hindrance, allowing for a more efficient interaction with fucoidan substrates (Cao *et al.*, 2018; Vuillemin *et al.*, 2020; Mikkelsen *et al.*, 2023). These findings offer valuable insights for engineering fucoidanases with improved catalytic properties.

This review aims to compile and critically analyze recent scientific advancements on the structural and functional characteristics of fucoidanases derived from marine microorganisms. It highlights the diversity in their catalytic mechanisms and addresses current challenges in enzymatic fucoidan degradation, including substrate specificity, enzyme stability, and the limited functional characterization of accessory domains. By deepening our understanding of the relationship between enzyme architecture and catalytic performance, these insights lay a solid foundation for future research aimed at the rational design and optimization of fucoidanases for targeted biotechnological and biomedical applications.

Diversity, sources, and catalytic properties of fucoidanases from marine microorganisms

Fucoidanases have been identified in a wide range of organisms, including marine

bacteria, marine fungi, sea cucumbers, sponges, and molluscs (Sakai *et al.*, 2003; Descamps *et al.*, 2006; Silchenko *et al.*, 2014). Among these, marine microorganisms–particularly those associated with brown macroalgae–are recognized as the primary source of fucoidanases discovered to date. The majority of these enzymes originate from marine bacterial strains, particularly those belonging to families such as Flavobacteriaceae, Alteromonadaceae, and Pseudoalteromonadaceae, which were commonly associated with marine organisms, especially brown seaweed (Kusaykin *et al*., 2015; Mikkelsen *et al.*, 2023). In contrast, fucoidanases from marine fungi were relatively rare, with only a few identified examples, such as fucoidanase LD8 from *Fusarium* sp. LD8 and TM94 from *Dendryphiella arenaria* TM94 (Wu *et al.*, 2011a, Wu *et al.,* 2011b).

In the past, fucoidanases were primarily screened and extracted directly from native marine microorganisms. Advances in genomics, bioinformatics, and recombinant protein expression have since shifted the focus towards genome mining and heterologous expression. Recombinant fucoidanases are now routinely identified from genome sequences of potential fucoidan-degrading strains. Notably, a single bacterial genome may encode multiple distinct fucoidanases, reflecting functional diversity and potential adaptation to structurally varied fucoidans. For instance, homologous fucoidanases such as Fhf1 and Fhf2 from *Formosa haliotis*, Mef1 and Mef2 from *M. eckloniae*, and FFA1 and FFA2 from *F. alga* have been reported (Mikkelsen *et al.*, 2023; Silchenko *et al.*, 2017a; Silchenko *et al.*, 2017b; Tran *et al.*, 2022; Vuillemin *et al.*, 2020). Interestingly, the genome of *Wenyingzhuangia fucanilytica* CZ1127^T revealed homologous fucoidanases belonging to three different CAZy families: GH107 (FWf1, FWf2), GH168 (FunA), and GH174 (AXE80-08890), highlighting the genetic diversity of fucoidan-degrading enzymes within a single bacterial strain (Table 1).

Table 1. Fucoidanases from marine microorganism.

Fucoidanase	Family in CAZy	Source	Strain	Characteristic				Reference
				Mode of action	Active conditions			
					pH	°C	NaCl (mM)	
Native fucoidanase								
LD8	-	Fungi	*Fusarium* sp. LD8	Endo-acting	6.0	50	-	Wu *et al*., 2011a
TM94	-	Fungi	*Dendryphiella arenaria TM94*	Endo-acting	6.0	50	-	Wu *et al*., 2011b
FcnA	GH107	Bacteria	*Mariniflexile fucanivorans* SW5^T	Endo-α-(1→4)	7.5	20-25	-	Descamps *et al.*, 2006
Fda1	GH107	Bacteria	*Alteromonas* sp. SN-1009	Endo-α (1→3)	6.5-8.0	30-35	-	Sakai *et al.*, 2004
No5	GH107	Bacteria	*Vibrio* sp. N-5	-	6.0-7.5	40	-	Furukawa *et al.*, 1992
Recombinant fucoidanase								

FFA1	GH107	Bacteria	*Formosa algae*	Endo-α-(1→4)	6.5-9.1	37	-	Silchenko *et al.*, 2017a
FFA2	GH107			Endo-α-(1→4)	6.5-9.1	25-37	-	Silchenko *et al.*, 2017b
FunA	GH168	Bacteria	*Wenyingzhuangia fucanilytica* CZ1127^T	Endo-α (1→3)	8.0	40	0-500	Shen *et al.*, 2020
FWf1	GH107			Endo-α-(1→4)	6.4-7.2	24-35	100-300	Zueva *et al.*, 2020
FWf2	GH107			Endo-α-(1→4)	6.0-6.8	24-40	100-300	Zueva *et al.*, 2020
AXE80-08890	GH174			Endo-α-(1→4)	7.0	37	-	Mikkelsen *et al.*, 2023
Fhf1	GH107	Bacteria	*Formosa haliotis*	Endo-α-(1→4)	8.0	37-40	100	Vuillemin *et al.*, 2020
Fhf2	GH107			Endo-α-(1→4)	8.0-9.0	35-45	100	Trang *et al.*, 2022
tFda1B	GH107	Bacteria	*Alteromonas* sp. SN-1009	Endo-α (1→3)	7.0	35	100	Zhu *et al.*, 2021
Mef1	GH107	Bacteria	*Muricauda eckloniae*	Endo-α (1→4)	7.0-9.0	20-37	25-400	Mikkelsen *et al.*, 2023
Mef2	GH107			Endo-α (1→3)	8.0	35	20-400	Tran *et al.*, 2022

(-): not study

Fucoidanases from marine microorganisms generally exhibit strict substrate specificity, most commonly toward α(1→3) and α(1→4) glycosidic linkages, or mixed α(1→3,4) arrangements. Their catalytic activity is strongly influenced by the sulfation pattern and branching complexity of the fucoidan backbone, which can either facilitate or hinder enzymatic accessibility. Marine-derived fucoidanases display remarkable diversity in catalytic specificity (Kusaykin *et al.*, 2015). In some cases, enzymes from phylogenetically distinct bacterial strains are able to hydrolyze the same type of glycosidic bond (Silchenko *et al.*, 2017a; Zueva *et al.*, 2020). Conversely, within a single bacterial strain, multiple fucoidanases can display distinct specificities toward different linkage types. For example, the marine bacterium *M. eckloniae* produces two fucoidanases, Mef1 and Mef2, that differ in catalytic preference: Mef1 selectively hydrolyzes α(1→4) linkages, whereas Mef2 targets α(1→3) linkages (Tran *et al.*, 2022; Mikkelsen *et al.*, 2023). Such specificity variation, even within the same organism, underscores the evolutionary adaptation of fucoidan-degrading systems to the structural heterogeneity of natural fucoidans.

Building on their remarkable diversity in substrate specificity, fucoidanases from marine microorganisms also display substantial variability in catalytic properties and optimal reaction conditions. Enzymes derived from marine fungi such as *Fusarium* sp. LD8 and *D. arenaria* TM94 were endo-acting, with optimal activity at pH 6.0 and 50°C, but differ in thermal stability, as *Fusarium* sp. LD8 remains active after incubation at 50°C for one hour (Wu *et al.*,

2011a, Wu *et al*., 2011b). In contrast, most marine bacterial fucoidanases exhibit optimal activity at lower temperatures (20–37°C), with a few exceptions reaching 40 to 45°C. Their pH preferences range from slightly acidic to alkaline, and their activity was influenced by NaCl concentration, with most remaining functional in saline environments but inhibited at high salt levels (Trang *et al*., 2022; Vuillemin *et al*., 2020; Zueva *et al*., 2020). These variations in catalytic properties highlight the adaptability of fucoidanases to diverse environmental conditions, making them promising candidates for industrial applications, such as targeted fucoidan degradation for functional oligosaccharide production, bioremediation, and pharmaceutical development.

Molecular insights into fucoidanase structure, catalytic domains, and functional adaptations

In recent years, advances in bioinformatics and genomic analysis have resulted in more and more sequences of fucoidanases being discovered (Vickers *et al.*, 2018; Vuillemin *et al.*, 2020; Mikkelsen *et al.*, 2023). The elucidation and structural analysis of the three-dimensional (3D) crystal structures of the marine bacterial fucoidanase MfFcnA have revealed that GH107 fucoidanases share a conserved N-terminal $(\beta/\alpha)_8$-barrel domain of approximately 400 amino acids, called the catalytic D1 domain, which functions as the active unit. The two catalytic amino acids, aspartate (D) and histidine (H) residues (Asp226 acts as a nucleophile and His294 acts as an acid/base catalyst) and four amino acids constituting of -1 subsite (Tyr147, Asn149, Asn270, and Trp351) of this family were predicted in the MfFcnA sequence that was known as the fucoidanase template sequence (Vickers *et al.*, 2018).

Building upon these structural insights, we conducted a comparative sequence analysis of GH107 fucoidanases to further explore their conserved catalytic features. Specifically, in this study, we compared the amino acid sequences of the conserved regions around the D1 catalytic domain and the -1 subsite of 11 GH107 fucoidanases from marine microorganisms, including three endo-α(1→3)-fucoidanases and eight endo-α(1→4)-fucoidanases (Table 2). The results revealed that all studied sequences exhibited fully conserved D and H residues, which were essential for their catalytic activity. Notably, these residues played a crucial role in glycosidic bond cleavage, ensuring enzymatic efficiency across different bacterial species. Additionally, the -1 subsite contained tyrosine (Y), asparagine (N), and tryptophan (W) as key residues, which facilitate substrate binding and specificity. However, it was noteworthy that the two N residues within the -1 subsite are not conserved across all GH107 sequences. For instance, variations in these residues have been observed in the endo-α(1→3)-fucoidanases Fda1 and Fda2 from the marine bacteria *Alteromonas* sp., as well as Mef2 from *M. eckloniae*. This variation suggests a potential role in determining the substrate specificity differences among fucoidanases; however, further statistical analysis and molecular studies are required to clarify this hypothesis.

Structurally, marine bacterial fucoidanases typically possess a GH107 catalytic D1 domain forming a $(\beta/\alpha)_8$ (TIM)-barrel fold, often accompanied by accessory domains such as galactose-binding-like domains, invasin/intimin-like adhesion modules, and in Bacteroidetes, a C-terminal T9SS secretion-

sorting signal. These accessory domains may modulate substrate binding, stability, or cell-surface localization rather than directly participating in catalysis (Vickers *et al.*, 2018; Vuillemin *et al.*, 2020; Trang *et al.*, 2022; Mikkelsen *et al.*, 2023) (Figure 1). Using the SignalP service, an N-terminal signal peptide has been identified in all GH107 members. Furthermore, other conserved domains in GH107 enzymes have been predicted using the InterProScan or the Conserved Domain Database (CDD) from the National Center for Biotechnology Information (NCBI). Among these, the cadherin-like domain (IPR015919) with an immunoglobulin-like beta-sandwich fold (Ig-like) is the most common domain found in the GH107 family. This domain, regularly located near the D1 domain, is present once in FWf1, FWf2 (Zueva *et al.*, 2020), Fhf1 (Vuillemin *et al.*, 2020), and Fhf2 and repeatedly in MfFcnA (Colin *et al*., 2006), FFA1 (Silchenko *et al*., 2017a), FFA2 (Silchenko *et al*., 2017b) fucoidanase sequences. The other common domain of GH107 fucoidanases was the type IX secretion system (T9), which functions to recognize or bind to substrates (Lasica *et al*., 2017). This domain is normally located at the C-terminal end of fucoidanase sequences, such as FWf1, FWf2, FWf3, FWf4 (Zueva *et al*., 2020), MfFcnA (Colin *et al*., 2006), FFA1 (Silchenko *et al.*, 2017a), FFA2 (Silchenko *et al*., 2017b), Fhf1 (Vuillemin *et al*., 2020), and Fhf2 fucoidanases. Furthermore, a β-strand domain was found in Mef2 and MfFcnA, and an SprB domain was found in Fhf1 (Vuillemin *et al*., 2020). In which, SprB was described as a cell surface protein involved in gliding motility in the bacterium *Flavobacterium johnsoniae* (Nelson *et al*., 2008), while the function of the β-strain domain is still unknown (Zueva *et al*., 2020).

Interestingly, while these conserved domains contribute to enzyme architecture, recent studies indicate that the extended C-terminal regions of GH107 fucoidanases may not be essential for catalytic activity. Truncation experiments on MfFcnA, Fhf1, Fhf2, and Fda1 have demonstrated that removing large portions of their C-terminal domains results in stable, heterologously expressed enzymes that retain catalytic activity. For instance, MfFcnA2, a truncated form of MfFcnA lacking the C-terminal T9SS domain, exhibited higher expression levels and stability compared to the full-length protein. Similarly, Fhf1Δ470 and Fhf2Δ484, which retain only the catalytic D1 and IgR domains, were more stable and functionally comparable to their native counterparts (Cao *et al.*, 2018; Vickers *et al.*, 2018; Vuillemin *et al.*, 2020).

Apart from domain architecture, other molecular factors such as metal ion interactions also play a crucial role in enzyme function. Calcium ions (Ca^{2+}) have been shown to influence fucoidanase activity significantly (Kusaykin *et al.*, 2015). Structural studies on MfFcnA and Mef1 by Vickers *et al*. (2018) and Tran *et al*. (2022) identified precise calcium-binding sites, highlighting their essential role in enzyme stability (Vickers *et al*., 2018; Tran *et al*., 2022). In GH107 fucoidanases, Ca^{2+} interacts with negatively charged residues, primarily D and glutamate (E), reinforcing structural integrity and maintaining catalytic efficiency under diverse environmental conditions. These findings suggest that calcium binding was a conserved feature among marine fucoidanases, contributing to their adaptation in marine ecosystems.

Table 2. Conserved regions around the D1 catalytic domain and the -1 subsite of GH107 marine bacterial fucoidanases.

Fucoid anases	Specificity	Conserved regions around the D1 catalytic domain and the -1 subsite											
Mef2	α-(1→3)	127	YISTQ	147	WDQFV	177	DGYWLD	199	DPTAVVTTN	255	DFTNGH	307	WH
Fda1	α-(1→3)	135	YIATQ	184	WRDYV	221	DGWWFD	244	NNDAAVAFN	268	DYTFGH	323	WN
Fda2	α-(1→3)	198	YIATQ	253	WRDYV	290	DGWWFD	313	NSNAAVSLN	337	DFTGGH	392	WN
MfFcnA	α-(1→4)	147	YVNSY	172	WMEWC	221	DAWCFD	262	NPNAAIAFN	289	DYTFGH	351	WN
Mef1	α-(1→4)	128	YIATD	150	WENYY	182	DGYWLD	207	DPSVMIASN	265	DFTSGH	318	WT
Fhf1	α-(1→4)	142	YVNSY	167	WEEYC	218	DAWCFD	258	NPNAAISFN	285	DYTFGH	364	WN
Fhf2	α-(1→4)	144	YVNSA	170	WKAYC	222	DAWCFD	262	NPNAAITFN	292	DYKFGH	355	WN
FFA1	α-(1→4)	144	YVNSY	169	WMEWC	218	DAWCFD	259	NPKAAISFN	286	DYTFGH	348	WN
FFA2	α-(1→4)	154	YVNSA	180	WKAYC	232	DAWCFD	272	NPDAAITFN	302	DYKFGH	365	WN
FWf1	α-(1→4)	129	YVNSS	156	WKEWC	220	DAWCFD	259	NPDAALSFQ	288	DFMFGH	357	WN
FWf2	α-(1→4)	365	YVNSS	397	WKAYC	459	DAWVFD	498	NENAAVAFN	532	DFMFGH	611	WQ

Two active sites in red letters: aspartate (D) and histidine (H); four suggested amino acids of the -1 subsite in blue letters: tyrosine (Y), two asparagine (N), tryptophan (W). The non-conserved amino acids of -1 subsite were in the blue letters with underline. The other conserved amino acids in all sequences were indicated in purple letters. The numbers indicated the position of amino acids in the corresponding sequences. Accession numbers were listed in method section.

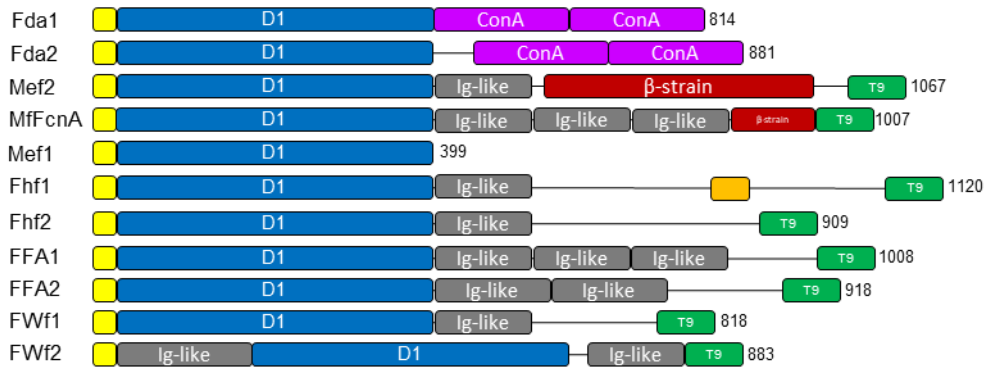

Figure 1. Primary structure and predicted domains of GH107 fucoidanases from marine bacteria.

While GH107 fucoidanases have been extensively studied, other fucoidanase families, such as GH168 and GH174, exhibit distinct molecular features that shape their catalytic mechanisms. GH168 enzymes, commonly found in *Vibrio* and *Alteromonas* species, possess extended loops that may enhance interactions with highly sulfated fucoidans. Despite significant progress in characterizing these enzymes, their precise catalytic region remains incompletely defined. However, Shen *et al.* (2020) identified two strictly conserved amino acids, D206 and E264, in FunA from the marine bacterium *W. fucanilytica* and its homologous sequences across the GH168 family. Site-directed mutagenesis in *E. coli* confirmed their essential role, as substitution of these residues led to a complete loss of enzymatic activity, strongly suggesting that D and E residues were key catalytic residues of GH168 fucoidanases (Shen *et al.*, 2020). Building upon these findings, recent studies have identified three additional key amino acids—D119, E120, and E218—as crucial for the activity of GH174 fucoidanases, reinforcing the importance of specific conserved residues in GH168 function.

Despite these advancements, the complete molecular structure of fucoidanases from marine microorganisms remains to be fully elucidated. Nevertheless, based on their enzymatic properties and sequence homology, it was likely that these enzymes possess unique catalytic domains and structural adaptations that enable broader substrate recognition and enhanced activity toward diverse fucoidan types.

CONCLUSION

In summary, this review emphasizes the structural features of fucoidanases from marine microorganisms and how they underpin catalytic activity and substrate specificity. Conserved catalytic residues, modular domain architectures, and calcium-binding motifs are critical for enzyme stability and efficiency in marine environments. Although recent X-ray crystallographic studies have provided important insights into active-site topology and domain functions—such as C-terminal truncation enhancing substrate accessibility—the number of structurally characterized fucoidanases remains limited. This scarcity, coupled with the high heterogeneity of fucoidan substrates, continues to present major challenges for enzyme classification and structure–function analysis. Future research should

therefore focus on expanding structural datasets and developing engineered fucoidanases with tailored activity, enabling broader applications in pharmaceuticals, biotechnology, and functional food industries.

ACKNOWLEDGMENTS

This work was funded and supported by a grant from the Vietnam Academy of Science and Technology (Grant no. VAST02.01/24-25).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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