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EDITED BY
Sachin Kumar,
Sardar Swaran Singh National Institute of Renewable Energy, India

REVIEWED BY
Diana Pacheco,
University of Coimbra, Portugal
Sasi Nayar,
South Australian Research and Development Institute, Australia

*CORRESPONDENCE
Noora Barzkar
noora.barzkar@gmail.com

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A recent update on fucoidonase: source, Isolation methods and its enzymatic activity

Noora Barzkar[1]*, Vilai Rungsardthong[1], Saeid Tamadoni Jahromi[2], Qandeel Laraib[3], Rakesh Das[4], Olga Babich[5] and Stanislav Sukhikh[5]

[1]Department of Agro-Industrial Technology, Faculty of Applied Science, Food and Agro-Industrial Research Center, King Mongkut's University of Technology North Bangkok, Bangkok, Thailand, [2]Persian Gulf and Oman Sea Ecology Research Center, Iranian Fisheries Sciences Research Institute, Agricultural Research Education and Extension Organization (AREEO), Bandar Abbas, Iran, [3]Department of Biosciences, Mohammad Ali Jinnah University, Karachi, Pakistan, [4]Department of Paraclinical Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences (NMBU), Ås, Norway, [5]Research and Education Center "Industrial Biotechnologies", Immanuel Kant Baltic Federal University, Kaliningrad, Russia

Fucoidanases are hydrolytic enzymes that degrade fucoidan to a lower molecular weight while retaining the side substituent groups of the polymer. Fucoidanases are produced by marine organisms: bacteria, fungi, algae, molluscs, echinoderms. Fucoidanases are rare and little studied enzymes. There is currently no information on the structural organization of fucoidanases, the size of active centers, their secondary and tertiary structures. This review summarizes the data on fucoidanase sources and factors influencing fucoidanase activity. It was found that that such factors include medium pH, temperature, and the presence of metal ions. The principles of classification of fucoidanases were analyzed. Fucoidanase was found to have high biological activity. Fucoidanases are known to hydrolyze fucoidan to oligosaccharides that have anti-inflammatory, antiangiogenic, anticancer, antiviral, prebiotic, and anticoagulant properties. Thus, research into sources, isolation methods, the effect of fucoidanase on fucoidan, and its enzymatic activity is promising, and can be used to build the body's resistance to adverse environmental factors (difficult working conditions, stress, and overwork), as well as restore and stimulate the immune response.

KEYWORDS

brown seaweed, fucoidan, fucoidanase, biological activity, hydrolysis, polysaccharides, fermentation mechanism

1 Introduction

In nature, algae are an ancient form of existing for photosynthetic organisms that belong to the plant kingdom and make up its largest group. Along with that, they constitute a considerable part of human diet in many parts of the globe, mainly in Southeast Asian countries. Since prehistoric times, seaweeds have been widely used as an alternative

medicine for different purposes. Besides, they are a source of polysaccharides with potentially active biological compounds. Brown algae fucoidan, is the most widely studied type of polysaccharide so far (Ale and Meyer, 2013; Ermakova et al., 2015; Biancarosa et al., 2018; Quitério et al., 2021). However, alginate is the most used polysaccharide in the industry. In general, the brown algae *Ectocarpales* and *Laminariales* can evolve polysaccharides with a structure consisting of 1 → 3-α-L-Fucp residues. Fucales is the main chain of fucoidan within the brown alga; it consists of linked 1 → 3 and 1 → 4 residues of α-L-fucoidan (Kusaykin et al., 2008; Holtkamp et al., 2009; Usov and Bilan, 2009; Hentati et al., 2020; Shiau et al., 2022). It (fucoidan) has a higher degree of polymerization. Therefore, depolymerization is required for fucoidan before medical or downstream applications. Enzymes that degrade polysaccharides have practical application in science, i.e. in structural studies, bioactivity studies, and drug manufacturing industries (Kusaykin et al., 2006; Drira et al., 2021).

Fucoidan is exposed to hydrolyze of some chemicals such as acids and bases, but enzymatic hydrolysis by fucoidanase is believed to be the main functional process involved in various biological activities towards sulfated polysaccharides. Till date, fucoidanase enzyme has only been described in a few or limited studies. It might be due to several factors, including a small number of a quantitative method for determining fucoidanase hydrolyzing activity, as well as the study's use of structurally uncharacterized or partially purified fucoidan as a substrate (Hentati et al., 2020; Drira et al., 2021).

Fucoidanases are a collective name for the enzymes involved in the hydrolysis of polysaccharides and fucoidan. This group includes fucoidanase (EC 3.2.1.44, α-L- fucosidase, poly(1,2-α-L-fucoside-4-sulfate) glycanohydrolase), an endoenzyme that catalyzes the hydrolysis of the (1→2)-α-L-fucoidan bond by retaining sulfate in fucoidan, while alpha-L-fucosidase (EC 3.2.1.51) contain L-fucose and α(1→ 3)-, α(1→ 4)-, α(1→ 2)-O-fucosyl bond. Various microbial fucoidanases promoted the degradation of algal fucoidan from different sources, resulting in a variety of degradation patterns and product structures. Enzymatic hydrolysis of fucoidan is generally considered to be an advanced sustainable green technology because it does not allow to hydrolyze fucoidan in chemically (Sørensen et al., 2007; Dobrinčić et al., 2020; Mikkelsen et al., 2021).

Such studies need to consider the structural diversity of fucoidan. In the case of source, bacteria that can hydrolyze fucoidan molecules has been under investigation since 1959 (Yaphe and Morgan, 1959) when Fucoidanase was discovered in the *Haliotis* sp. (Thanassi and Nakada, 1967; Zayed and Ulber, 2020). In addition to the marine bacteria (Bakunina et al., 2000; Descamps et al., 2006; Silchenko et al., 2013; Trang et al., 2022) and marine invertebrates (Kitamura et al., 1992; Daniel et al., 1999; Kusaykin et al., 2003; Bilan et al., 2005), fucoidanase was also found in some listed fungi (Rodríguez-Jasso et al., 2010; Cui et al., 2018). Though, fucoidanase obtained from such agents, is comparatively far less common that the other enzymes like glycosidases produced from cellulases, amylases, laminarinases. Fucoidanases (formerly known as α-L-fucosidases) can be extracted as an intracellular as well as an extra-cellular metabolite. The information concerning the fermentative power of fucoidanases i.e. site of dissociation of glycoside bond in fucoidan, the coalition in their catalytic action, can rarely be found. Even though the sequence of genes affecting the structure of fucoidanase has been reported, and the specific amino acid structure of fucoidanases isolated from *Mariniflexile fucanivorans* SW5 (Silchenko et al., 2013) and *Alteromonas* sp. SN-1009, however, endofucosidase does not yet exist in the commercial form. Since, fucoidanases are less studied enzymes therefore, actual mode of action is still unclear (Birolli et al., 2019).

Hence, the present article has been designed to highlight the fucoidans, generated by specifically brown seaweed, along with to discuss new information or data on the fucoidanase enzyme hydrolyzing fucoidan, as well as to determine its properties and biological activity for possible downstream applications.

2 Sources of fucoidanase and its enzymatic activity

To date, enzymes that decompose fucoidan are produced by a wide range of living organisms (marine bacteria, seaweed, sea urchins). The fucoidanase enzyme was produced by marine bacteria, seaweeds, and sea urchins as a result of the formation of fucoidan during their vital activity, which is cleaved by fucoidanase to ensure the metabolism of these organisms. List of organisms producing fucoidannase are presented in Table 1.

To date, there are several studies have highlighted about the isolation and characterization of fucoidanase including various possible methods for isolating and purifying fucoidanase. The general protocols followed for isolation and purification of some fucoidanases producing organisms from different marine sources are summarized in the Table 2.

So far, different marine ecosystems such as Busan coast at South Korea (Manivasagan and Oh, 2015); Aomori at Japan (Sakai et al., 2004); Sewha Beach, Jeju Island at Korea (Kim et al., 2008); Baltic Sea and North Sea at Germany (Qianqian et al., 2011; Wu et al., 2011) have been studied with the aim to determine isolation of fucoidanases producing by marine organisms. Mentioned bacteria and fungi producing fucoidanase enzymes have been isolated from marine sediment (Manivasagan and Oh, 2015), seawater (Sakai et al., 2004), brown algae *F. evanescens* L. (Silchenko et al., 2013), sea sand (Furukawa et al., 1992; Qianqian et al., 2011; Wu et al., 2011), and mollusks and sea urchin producing fucoidanase enzymes have been isolated from digestive glands (Silchenko et al., 2014), hepatopancreas (Kitamura et al., 1992), and digestive tract (Sasaki et al., 1996).

2.1 Fucoidanase from marine bacteria

In another finding, a marine isolated such as *Fucophilus fucoidanolyticus* L. has been reported to synthesize numerous types of fucoidan-degrading enzymes in that way they were proficient in utilizing fucoidans of various structures. Additionally, intracellular fucoidanase also demonstrated the ability to hydrolyze 1,3- α-glycosidic bonds and to exhibit deacetylase activity on acetylated fucoidans produced by *Cladosiphon okamuranus* Tokida (Sakai et al., 2004).

Fucoidanase was preliminarily isolated from several marine microbial florae namely, *Vibrio* sp., *Alteromonas* sp.,

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TABLE 1 Fucoidanase-producing organisms.

Organisms	Type of organism	Source
Vibrio sp. N-5	marine bacteria	(Furukawa et al., 1992; Bilan et al., 2006; Usoltseva et al., 2019; Choi et al., 2022; Lin et al., 2022)
Fucobacter marina SI-0098	marine bacteria	(Wang et al., 2008)
Pseudoalteromonas citrea KMM 3296	marine bacteria	(Bakunina et al., 2002)
Pseudoalteromonas citrea KMM 3297	marine bacteria	(Bakunina et al., 2002
Pseudoalteromonas citrea KMM 3298	marine bacteria	(Bakunina et al., 2002
Fucobacter marina SA-0082	marine bacteria	(Wang et al., 2008)
Fucophilus fucoidanolyticus SI-1234	marine bacteria	(Bakunina et al., 2002; Urvantseva et al., 2006)
Mesonia algae KMM 3909	marine bacteria	(Urvantseva et al., 2006)
Maribacter sp. KMM 6211	marine bacteria	(Urvantseva et al., 2006)
Gramella sp. KMM 6054	marine bacteria	(Ohshiro et al., 2012)
Alteromonas sp. SN-1009	marine bacteria	(Yarza et al., 2014)
Luteolibacter sp. H18	marine bacteria	(Nagao et al., 2018)
Mariniflexile fucanivorans SW5	marine bacteria	(Silchenko et al., 2013)
Sphingomonas paucimobilis PF-1	marine bacteria	(Silchenko et al., 2013)
Formosa KM3553	marine algae	(Silchenko et al., 2013)
Fusarium sp. LD8	marine fungi	(Qianqian et al., 2011)
Pseudomonas atlantica	marine bacteria	(Vuillemin et al., 2020)
Pseudomonas carrageenovora,	marine bacteria	(Vuillemin et al., 2020)
Formosa	marine algae	(Vuillemin et al., 2020)
Streptomyces sp.	marine bacteria	(Manivasagan and Oh, 2015)
Pseudoalteromonas sp. SB 1493	marine bacteria	(Lee et al., 2012)
Sphingomonas paucimobilis PF-1	marine bacteria	(Kim et al., 2015)
Dendryphiella arenaria TM94	marine fungi	(Bilan et al., 2002; Bilan et al., 2004; Bilan et al., 2006; Rodríguez-Jasso et al., 2010; Wu et al., 2011; Ahmad et al., 2022; Choi et al., 2022; Iliou et al., 2022), (Foley et al., 2011), (Wu et al., 2011)
Lambis sp.	marine mollusk	(Silchenko et al., 2014)
Littorina kurila	marine mollusk	(Kusaykin et al., 2003; Bilan et al., 2005; Yang et al., 2021b)

(Continued)

TABLE 1 Continued

Organisms	Type of organism	Source
Haliotus sp.	marine mollusk	(Thanassi and Nakada, 1967)
Mizuhopecten yessoensis (Patinopecten yessoensis)	marine mollusk	(Kitamura et al., 1992)
Vasticardium flavum	marine mollusk	(Khanh et al., 2019b)
Echinodermata	echinoderms	(Chevolot et al., 2001; Larsson et al., 2003; Bilan et al., 2004; Bilan et al., 2005; Yang et al., 2021b; Abdel-Latif et al., 2022; Ahmad et al., 2022)
Strongylocentrotus nudus	Sea urchins	(Sasaki et al., 1996)

Pseudoalteromonas sp., and the members of *Flavobacteriaceae* (Furukawa et al., 1992; Shin-Ichi et al., 1992; Bakunina et al., 2000; Descamps et al., 2006). Subsequently, 25 epiphytic bacterial cultures isolated from brown algae, as well as more than 53 sea cucumber strains were screened and showed a significant result in fucoidanase production. The highest rate of fucoidanase activity was noted in *Cytophaga* and *Alteromonas/Pseudoalteromonas* species (Bilan et al., 2005; Yang et al., 2021a).

Furthermore, the novel marine bacterium *Fucanobacter lyticus* is an effective indicator of extracellular and intracellular fucoidanase causing degradation of fucoidan isolated from brown algae belonging to the genera *Fucales* and *Chordariales* and found to be effective for hydrolysis of fucoidan. Thus, *Chordariales* demonstrated the specificity of endo-1,3-α-fucoidanase (Sakai et al., 2004).

For example, fucoidanase from marine bacteria strain SN-1009 was purified using DEAE-Cellulofine A-800, DEAE-Sepharose FF, Sephacryl S-200, Phenyl-Sepharose CL-4B, and DEAE-Cellulofine A-800 column chromatography (Sakai et al., 2004). The purified enzyme showed specific activity of 2730 (mU/mg), and its output was 12%; the enzyme had molecular weight of 100 kDa (Sakai et al., 2004; Silchenko et al., 2014). Marine mollusk *Lambis* sp. produced fucoidanase using a five-step procedure in this case fuconoidase was isolated from Phenyl-sepharose, Sephacryl S-100, DEAE-MacroPrep, and CM-MacroPrep, TSK 2000 after concentration with ammonium sulfate precipitate. The enzyme demonstrated specific activity of 2.571 U/mg, and the purification efficiency was 99 times higher (Silchenko et al., 2014).

Bioactive fucoidan oligosaccharides obtained by the fucoidanase action are one of the promising components for biomedical applications. The high biological activity of fucoidans depends on the degree of their sulfation. In the study (Trang et al., 2022), scientists identified a new fucoidanase Fhf2 isolated from the genome of the marine bacterium *Formosa haliotis*. Thus, Fhf2 was found to be similar to endo-α(1,4)-fucoidanases (EC 3.2.1.212) of the glycoside hydrolase class.

2.2 Fucoidanase from seaweed

According to the study (Tran et al., 2022b), endofucoidanases such as endo-α-1,3-L-fucanase EC 3.2.1.211 and endo-α-1,4-L-fucanase EC 3.2.1.212 catalyze the depolymerization of fucoidans. Quantitive evaluation of endofucoidanase activity is of exceptional importance for characterizing the endofucoidanase kinetics and for comparing the effects of different endofucoidanases on various types of fucoidans. The study used the Fourier Transform Infrared Spectroscopy (FTIR) along with the sensitive factor analysis to quantify endofucoidanase. The results coincide with the data obtained by monitoring microbial enzymes FcnAΔ229, FFA2 and Fhf1Δ470 on fucoidan substrates. These substrates were isolated from the algae *Fucus evanescens* L. and *Fucus vesiculosus* L. The results of the studies showed that the peak of the spectra of fucoidans during fermentation fell in the range of 1220–1260 cm^{-1}; however, their profiles on different substrates differentiated. Spectral peaks in the range 1220–1260 cm^{-11} correspond to the absorption spectra of sulfated fucosyls. In this range of the spectra, sulfate-ether bonds and stretching vibrations of CO-groups were absorbed. One unit of endofucoidanase activity or Uf is the amount of enzymes affecting the change in FTIR-PARAFAC by 0.01 in 498 seconds of reaction for 20g/l of pure fucoidan isolated from *F. evanescens* L. at 42°C, acidity 7.4, for solutions NaCl (100 mM) and $CaCl_2$ (10 mM). The proposed quantitative analysis of endofucoidanase reveals new possibilities for studying endofucoidanases (Tran et al., 2022b).

Endo-α(1,4)-fucoidanase with molecular weight of 46 kDa was found to be more stable than the Fhf2 enzyme. Fhf2 was used for fermentation of seaweed fucoidans including *F. evanescens* L., *F. vesiculosus* L., *Sargassum mcclurei* Setchell, and *Sargassum polycystum* C.Agardh. The highest enzymatic activity of Fhf2 was shown in relation to fucoidan from *F. evanescens* L. The enzyme Fhf2 was active at 20–45°C and pH 6–9. In addition, the activity of this enzyme solely depended on the calcium content. NMR analysis showed that Fhf2 hydrolyzes α(1,4) bonds between sulfated L-fucosyl residues and releases oligosaccharides with a large amount of 2,4-disulfated fucose residues. Thus, it can be concluded that the Fhf2 fucoidanase is promising for the isolation of highly sulfated oligosaccharides, which are planned to be used to study the biological activity of fucoidan (Trang et al., 2022).

In the study (Silchenko et al., 2017b), it was established that it was possible to clone the gene encoding fucoidanase FFA2 in strains KMM 3553 of seaweed *Formosa* and in the bacterium *Escherichia coli*. Recombinant fucoidanase FFA2 was purified and its

TABLE 2 Isolation and purification of some fucoidanase-producing organisms from various sources.

Organism	Source	Isolated from	Species	Concentration Method	Used chromatography column	Total Protein (mg)	Total Activity (U)	Mw (kDa)	Purification (-Fold)	Specific activity (U/mg)	Yield (%) d.w.	Ref
Bacteria	Busan coast (Lat 35°09′ N; Long129°07′ E), South Korea	Marine sediment	*Streptomyces* sp.	$(NH4)_2SO_4$ precipitation	Sephadex G-100 column	16.74	12.81	ND (Not Determined)			3.1	(Manivasagan and Oh, 2015)
	Aomori, Japan	Coastal seawater	Strain SN-1009	$(NH4)_2SO_4$ precipitation	DEAE-Cellulofine A-800, DEAE-Sepharose FF, Sephacryl S-200, Phenyl-Sepharose CL-4B, and DEAE-Cellulofine A-800	4.32	11,800 (mU)	100	3500	2,730 (mU/mg)	12	(Sakai et al., 2004)
	Sewha Beach, Jeju Island, Korea	Seawater	Strain PF-1	$(NH4)_2SO_4$ precipitation	Bio-Gel P-4 column	4.4	84.08	60-130	112.8	0.019 ($U{\cdot}mg^{-1}$)	13.6	(Kim et al., 2008)
	PIBOC* Marine Microorganisms Collection		*Luteolibacter algae* H18	$(NH4)_2SO_4$ precipitation	Resource Q column	ND (Not Determined)		112	25	ND (Not Determined)	-	(Nagao et al., 2018)
	PIBOC* Marine Microorganisms Collection	Brown algae *Fucusevanescens*	*Formosa algae* strain KMM 3553^T	Ultrafiltration	DEAE-MacroPrep column, Sephacryl S-200 column	ND (Not Determined)		20.7	33.4	ND (Not Determined)	9.4	(Silchenko et al., 2013)
	Nha Trang Bay, Vietnam		*Formosa haliotis*	Ethanol precipitation	Ion-exchange	ND (Not Determined)			71	ND (Not Determined)	0.98	(Vuillemin et al., 2020)
	NA (Not available)	Sea sand	*Vibrio* sp. N-5	–	–	ND (Not Determined)					–	(Furukawa et al., 1992)
Fungi	Baltic Sea in Germany	Sand	*Dendryphiellaarenaria* TM94	Acetone precipitation	Sephadex G-100	210.05	67.07 (IU)	180	26.67	0.32 ($IU{\cdot}mL^{-1}$)	17.69	(Wu et al., 2011)
	North Sea in German	Sand	*Fusarium* sp. LD8	Acetone precipitation	Sephadex G-100	120.43	30.64 (IU)	64	22.7	0.25 ($IU{\cdot}mg^{-1}$)	23.9	(Qianqian et al., 2011)
Mollusk	NA (Not available)	Digestive glands	*Lambis* sp.	$(NH4)_2SO_4$ precipitation	Phenyl-sepharose, Sephacryl S-100, DEAE-MacroPrep, CM-MacroPrep, TSK 2000	0.11	0.28	50	99.0	2.571	0.75	(Silchenko et al., 2014)
	Shoji Store Co. (Hokkaido)	Hepatopancreas	*Patinopectenyessoensis*	$(NH4)_2SO_4$ precipitation	DEAE-Toyopearl 650 M, Isoelectric focusing, Sephacryl S-30	6.2	24.2	84	14	3.9	27	(Kitamura et al., 1992)
Sea urchin	NA (Not available)	Digestive tract	*Strongylocentrotusnudus*	$(NH4)_2SO_4$ precipitation	SP-Toyopearl 650, CM -Sepharose FF, Sephacryl S-200	5.6	864	130	307	154	1.6	(Sasaki et al., 1996)

*- G.B. Elyakov Pacific Institute of Bioorganic Chemistry of the Far-Eastern Branch of the Russian Academy of Sciences.

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biochemical properties were studied. It was found that FFA2 has amino acid sequence identical to the one of fucoidanase FcnA isolated from *M. fucanivorans*. The molecular weight of the gene FFA2 was 101.2 kDa (918 amino acid residues). Analysis of this sequence showed that the studied fucoidanase FFA2 belongs to the class of glycoside hydrolases GH107 (CAZy). Substrate specificity was studied in details by using fucoidans from brown algae and synthetic fucooligosaccharides. Degradation of (1→4)-α-glycosidic bonds in the fucoidan of the brown alga *F. evanescens* L. FFA2 catalyzed within the fragment of fucoidan structure (→3) -α - L -Fuc p 2S-(1→4) -α - L -Fuc p 2S -(1→) n, but if the fragment structure was (→3) - α - L -Fuc p 2S,4S-(1→4) -α- L-Fuc p 2S-(1→) n, FFA2 enzymatic activity was absent.

2.3 Enzymatic activity of fucoidanases

Use of fucoidanase helps to assess the exact molecular structure responsible for the protective effect of biological activity. In addition, fucoidan-cleaving enzymes may help elucidate the various structures of fucoidan, paving the way for industrial applications of these enzymes.

To date, the activity of enzymes in relation to fucoidans has been little studied. The study (Vickers et al., 2018) presented an analysis of three enzymes from the class of glycoside hydrolases 107 (GH107): MfFcnA, P5AFcnA, and P19DFcnA, isolated from the culture liquid of bacteria of the genus *Psychromonas*. These enzymes were shown to be substrate-specific and active against fucoidans. Studies established that GH107 family enzymes share a common structure and catalytic mechanism with the GH29 α-l-fucosidase enzyme, the fact was proved by X-ray crystallography and NMR analysis. However, GH107 enzymes used the histidine side chain as an acid-base catalyst in the retention mechanism. Interpretation of the structure of the enzymes showed that these classes of enzymes differ in the architecture of the active sites for different substructures of fucoidan. According to the study (Vickers et al., 2018), these results illuminate the mechanism of molecular biological based processing of fucoidans.

3 Classification of fucoidanases

Due to the complex structure of fucoidans, which are heteropolysaccharides, fucoidanases are difficult to be classified correctly. Fragmentary data on the structures of fucoidans lead to an incorrect classification of fucoidanases. For example, fucoidan isolated from *F. vesiculosus* L. was determined incorrectly as a unit with structure 1→2-α-L-fucan. This definition led to the incorrect assignment of fucoidanases to EC 3.2.1.44. Subsequently, the structure of the enzyme was refined and assigned to α-1→3- and α-1→4 bond residues of fucose. However, there is a lack of knowledge for a complete and correct classification of fucoidanases. In addition, it is noted that enzymes that hydrolyze any glycosidic bonds in fucanes (polysaccharides from fucose residues) are called fucanases rather than fucoidanases (Kusaykin et al., 2016).

Comparison of amino acid sequences has led to an alternative classification of fucoidanases based on hydrolyzed bond types. The classification by carbohydrate active enzymes or CAZy (www.cazy.org) is called homologous. According to CAZy, glycoside hydrolase (GH) enzymes are divided into 133 classes (GH classes). Peptides with unknown properties can be found in each class, if they are similar in amino acid structure and sequence, they can also be classified by CAZy. CAZy can be used to predict the catalytic and molecular properties of fucoidanases (Gebler et al., 1992) and the structural geometry of cleavable glycosidic bonds (Henrissat et al., 1995). The CAZy system includes 133 GH classes united into 14 families of closely related classes. Due to the presence of sites that belong to several classes, some GHs are multifunctional enzymes. By applying this classification, fucoidanases can be assigned to class 107 GH. Fucoidanase FcnA isolated from the marine bacterium *M. fucanivorans* strain SW5T is one of the first members of this class. A comparative analysis of the secondary structure elements of the fucoidanase FcnA and other known fucoidanases subsequently made it possible to state that this entire class contains data on four sequences: FcnA (CAI47003.1) from *M. fucanivorans* SW5T, Fda1 (AAO00508.1) and Fda2 (AAO00509.1) from *Alteromonas* sp. SN-1009 and gene SVI_0379 (BAJ00350.1) from *Shewanella violacea* DSS12T (Kusaykin et al., 2016).

4 Factors influencing fucoidanase activity

4.1 pH influence

In Table 2 we can observe that fucoidanase of marine bacterium origin is most active in the neutral or slightly alkaline pH medium. Furthermore, fucoidanase isolated from the seaweed strain *Formosa* KMM3553 (Silchenko et al., 2013) showed supreme activity in an extensive range of acidity (pH: 6.5 to 9.1). Invertebrate enzymes usually have an optimal acidic pH, apart from the enzymes contained in the liver-pancreas of the marine mollusk *Littorina kurila* (Kusaykin et al., 2003). In marine bacterium, *Formosa algae* strain KMM 3553, two forms of fucoidanase were found in this organism, with acidic pH (about 5.5) and basic pH (about 8) being optimal (Table 3). Fucoidanase isolated from mollusca *Haliotus* sp. (Thanassi and Nakada, 1967), *Lambis* sp. (Silchenko et al., 2014), *Vasticardium flavum* (Khanh et al., 2019a), and echinus *Strongylocentrotus nudus* (Sasaki et al., 1996), showed supreme activity in the pH range of 3.5–5.5 (Table 2). There are only a few publications so far on marine fungal fucoidanase. Nowadays, scientists studied and characterized only fucoidanases isolated from *Dendryphiella arenaria* TM94 (Wu et al., 2011) and *Fusarium* sp. LD8 (Qianqian et al., 2011). It shows that fucoidanase of marine fungi origin showed supreme activity at pH 6.0 (Table 3).

Fucoidanase from the marine fungus *Dentryphiella renaria* TM94 is stable at pH 6–7; but when pH is 3.0 and 8.0, there is a loss of activity by 50% after 4 and 6 hours, respectively, and complete inactivation of fucoidanase is achieved at 100°C in 30 minutes (Wu et al., 2011). The most responsive to temperature changes appeared to

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TABLE 3 Physico-chemical properties of fucoidanase produced from various sources.

Fucoidanases source	Species	pH Opt	Temp Opt	pH stability	Temp stability (°C)	Inhibited by	Activated by	K_m (mg ml^{-1})	Vmax ($mg{\cdot}mL^{-1}{\cdot}min^{-1}$)	Ref
Bacteria	*Luteolibacter algae* H18	7.3	40	stable at pH 5.5-8.5 after incubation at 30°C for 30 min	retained more than 90% of its activity after incubation at 40° C for 30 min but enzyme activity was lost above 50°C	Co^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+}	Ba^{2+}, Fe^{2+}, Ca^{2+}, Al^{3+}	–	–	(Nagao et al., 2018)
	Formosa haliotis	8	37-40	–	–	–	Ca^{2+}, Mn^{2+}, Zn^{2+} or Ni^{2+}	–	–	(Vuillemin et al., 2020)
	*Pseudoalteromonas*sp strain SB 1493	8	50	–	–	–	–	–	–	(Lee et al., 2012)
	Formosa algae strain KMM 3553	6.5 to 9.1	–	–	Activity of fucoidanase was considerably reduced after prolonged (about 60 min) incubation of the enzyme solution at 45°C. The enzyme was completely inactivated after 40 min of incubation at 55° C	Cu^{2+} and Zn^{2+}	Mg^{2+}, Ca^{2+} and Ba^{2+}	–	–	(Silchenko et al., 2013)
	Vibrio sp. N-5	6	40	stable between pH 5 and 8	Completely inactivated over 70°C	–	–	–	–	(Furukawa et al., 1992)
	Formosa haliotis	8	37	–	Inactive at temperatures of 55°C and above.	–	–	–	–	(Vuillemin et al., 2020)
Fungi	*Dendryphiellaarenaria* TM94	6	50	3.0 and 8.0, an activity loss of about 50% occurred after 4 and 6 h, respectively	At 30°C the activity of fucoidanase decreased to 12.5%, while at 80°C to 18.75%. Fifty percent inactivation of the fucoidanase activity occurred at 56°C for 1 h, and total fucoidanase inactivation could be achieved at 100°C for 30 min.	–	–	6.56	6.55	(Wu et al., 2011)
	Fusarium sp. (LD8)	6	60	Displayed stability at pH 6.0, whereas at pH 5.0 and 8.0, an activity loss of about 50% occurred after 6 h incubation at room temperature (25°C), respectively.	half inactivation was 50°C	–	–	8.9	2.02	(Qianqian et al., 2011)

(Continued)

TABLE 3 Continued

Fucoidanases source	Species	pH Opt	Temp Opt	pH stability	Temp stability (°C)	Inhibited by	Activated by	K_m (mg ml^{-1})	Vmax ($mg{\cdot}mL^{-1}{\cdot}min^{-1}$)	Ref
Mollusk	*Lambis* sp.	4.9	37	–	half-inactivation time was 20 min at 54 °C	Hg^{2+}, Zn^{2+}, Cu^{2+}	Mg^{2+}, Ba^{2+}, Ca^{2+}	1.3	–	(Silchenko et al., 2014)
	Littorina kurila	5.4 and 8.5	–	–	–	–	–	–	–	(Kusaykin et al., 2003)
	Haliotis sp.	5.4	38	2-10	At 50°C about 85% of the activity remained. purified enzyme apparently resisted denaturation until the temperature was above 50	Hg^{++}, Ag^{+}	–	–	–	(Thanassi and Nakada, 1967)
	Vasticardium flavum	3-4	–	–	Activity was greatly reduced after 60 min of the denaturation at 45 °C.	Cu^{2+}, Sn^{2+}, Fe^{2+}, Al^{3+}	Ca^{2+}, Ba^{2+}, Co^{2+}, Mg^{2+}	–	–	(Khanh et al., 2019a)
Sea urchin	*Strongylocentrotus nudus*	3	45	2.0 to 5.0	below 50°C	–	–	–	–	(Sasaki et al., 1996)

be marine fungus *Fusarium* sp. LD8, as they loss half of their activity in about 1 hour at 50°C; although, and the enzyme had stable character at pH 6.0 (Qianqian et al., 2011) (Table 3).

4.2 Temperature influence

For marine invertebrate fucoidanase, the optimal temperature range is 38-45°C (Silchenko et al., 2014) (Kusaykin et al., 2003) (Thanassi and Nakada, 1967; Sasaki et al., 1996; Khanh et al., 2019a), and for marine bacteria and fungi it is 37-50°C (Furukawa et al., 1992; Lee et al., 2012; Silchenko et al., 2013; Nagao et al., 2018; Vuillemin et al., 2020) and 50-60°C (Qianqian et al., 2011; Wu et al., 2011). Marine bacterium *Vibrio* sp. N-5 produces fucoidanase that is completely inactivated at temperatures above 70°C (Furukawa et al., 1992); while marine bacterium *F. haliotis* gives fucoidanase inactivated at temperatures above 55°C (Vuillemin et al., 2020). These data indicates that fucoidanase can be characterized as active and stable in different medium with a varied range of pH; it is typical of fucoidanases isolated from the major part of marine sources as bacteria and invertebrate organisms (Silchenko et al., 2013). Besides, we found that this fucoidanase is functional even at high temperatures. Meanwhile, elevated temperature at industrial treatment and processing are considered prominent as this let reducing microbial contamination in large scale industrial reactions of prolonged durations (Ahmed et al., 2009; Garuba et al., 2020).

4.3 Influence of metal ions

Dependence of fucoidanase activity on impact of inhibitors and activators has not been thoroughly studied. Several publications devoted to the effect of metal ions on the fucoidanase activity. They include studies on divalent metal cations' impact on the enzymatic activity of fucoidanases of different origin: isolated from marine bacterium *Vibrio* sp. N-5 (Furukawa et al., 1992) and from marine mollusk *Haliotis* sp. (Thanassi and Nakada, 1967). The research showed that these enzymes do not depend on metals. Though, cations of some metals can affect fucoidanase activity; but they are inhibited by Ag^{+} and ions of divalent metals (Hg^{2+}, Fe^{2+}, and Mn^{2+}). Ions of Cu^{2+} and Pb^{2+} produce no effect on the activity of these enzymes, while ions of Co^{2+} and Mg^{2+} slightly activate the enzymes (Thanassi and Nakada, 1967; Furukawa et al., 1992). The activity of fucoidanase from *Vasticardium flavum* is enhanced by Ca^{2+}, Ba^{2+}, Co^{2+}, and Mg^{2+} cations, but inhibited by Cu^{2+}, Sn^{2+}, Fe^{2+}, and Al^{3+} cations (Khanh et al., 2019a). In one study (Nagao et al., 2018), the activity of the enzyme was intense by divalent cations such as Ba^{2+}, Fe^{2+}, and Ca^{2+}, while inhibited by Co^{2+}, Cu^{2+}, Mn^{2+}, and Zn^{2+} (Nagao et al., 2018) and *Formosa* KMM3553 fucoidanase requires metal ions for its activity (Silchenko et al., 2013) (Table 3). These facts propose that metal ions to have influence on either substrate binding or catalytic activity.

In consequences, fucoidanase enzyme activity primly depends on type of organisms and its physicochemical parameters. Hence, right identification of organism for the fucoidanase enzyme production is a need-based approach.

5 Biological activity of fucoidanases

Significant interest in health-promoting properties of polysaccharide bio-macromolecules of marine origin, particularly acidic polysaccharides, has been sparked by developments

particularly in biopharmaceutical and genetic engineering techniques. Fucoidan is one of these and is thought to be promising (Wang et al., 2019). The structure of these polysaccharides is so irregular that it is difficult to study them chemically and analyze their structure in detail (Sergeevich and Olegovna, 2015). To address this issue, specific enzymes capable to hydrolyze complex fucoidan molecules are required.

Fucoidanase catalyzes the O-glycosidic bond hydrolysis in the main chain of the fucoidan molecule, while exhibiting biological activity. Moreover, fucoidanase is a rare and little-studied enzyme. To date, there is no information or very scanty information on the complete structure of fucoidanase, active sites, substrate binding sites, secondary and tertiary structures. The mechanism of fucoidanase action has not yet been completely understood. Moreover, due to their heterogeneity, fucoidans are still largely underutilized as a base of pharmaceuticals despite having a wide range of biological activity and being non-toxic when taken orally (Kusaykin et al., 2016). Therefore, one possible solution to address such issue is to define the sequence of amino acid in fucoidanase and obtain enough recombinant enzymes to study the enzyme's properties and spatial structure (Tran et al., 2022a).

There was determined the sequence of amino acid in fucoidanase isolated from the marine bacterium *Formosa* algae. They were obtained by recording the nucleotide sequence of the *F. algae* genome and homologs of gene products contained in the genome of algae were found (Sergeevich and Olegovna, 2015). As a result, it can be concluded that, the algae genome contains two genes encoding fucoidanase (FFA1 and FFA2) and the review of the amino acid sequences of FFA1 and FFA2 by multiple alignment showed 57% identity. The amino acid sequence of FcnA is 67% and 57% identical for FFA1 and FFA2, respectively.

Cadherin-like domains were found in bacterial enzymes that accelerate decomposition of various compounds (proteins, carbohydrates, nucleic acids) and lectins that bind to anionic polysaccharides (Abdian et al., 2013). It should be noted that the functions of these domains described in the literature are common for binding to one or another biopolymer using calcium ions (calcium dependent binding).

The gene construct encodes full-length fucoidanases (FFA1 and FFA2) and their truncated derivatives without the C-terminal domain of FFA1-SD and FFA2-SD, and without the cadherin-like repeating domain of FFA2-KD and FFA1-KD, containing nucleotide sequences (Sergeevich and Olegovna, 2015).

The full-length recombinant fucoidanase and all its truncated derivatives catalyze the breakdown of fucoidan. The fucoidanase activity of recombinant and native enzymes is manifested only if there are ions of certain divalent metals. The presence of fucoidanase activity in recombinant FFA1-SD fucoidanase and truncated FFA2-SD derivatives suggests that the C-terminal domain may not be involved in catalysis. So, it could be stated that, fucoidanase FFA1-KD and FFA2-KD also have the ability to hydrolyze fucoidan, but to a much lesser extent. This modification of the protein can reduce its ability to bind to substrate molecules. If the cadherin-like domain plays a stabilizing role, then similar results can be achieved by decreasing the molecule stability (Abdian et al., 2013; Silchenko et al., 2013).

Nonetheless, both the enzymes rapidly hydrolyze fucoidan. The fucoidan chain alternates with residues of sulfated fucose that are linked to α-1 → 3 and α-1 → 4. The obtained data shows that both fucoidanases are specific for splitting of the α-1 → 4-glycosidic bond within residues of sulfated fucose (Tran et al., 2022a).

At present, three principal mechanisms of fucoidan modifying enzymes have been identified and listed below: a-L-fucosidase acts as a catalyst for the degradation of the a-L-fucosyl linkages at the nonreducing ends, thereby liberating fucose units from the major backbone of fucose in fucoidans (Figure 1).

While the other type of fucoidanase causes random endo-type hydrolysis of glycosidic linkages (both α (1→3)-, and α (1→4)-) found in the fucose (Figure 1).

The study (Silchenko et al., 2017a) observed the anticancer activity of fucoidan from *S. horneri* var. *densum* C.Agardh, described its structure and established the process of its transformation under the action of fucoidanases. The gene encoding fucoidanase from the marine bacterium *F. algae* was identified and cloned; subsequently FFA1 was generated in *Escherichia coli*. The product of gene FFA1 had a molecular weight of 111 kDa. Amino acid sequence analysis indicated that the FFA1 fucoidanase belongs to the class of glycoside hydrolases GH107 (CAZy). The resulting recombinant fucoidanase FFA1 was used to obtain fucooligosaccharides. NMR spectroscopy was used to determine the structure of 5 sulfated oligosaccharides with a degree of polymerization of 4–10. It was shown that the fucoidan extracted from *S. horneri* var. *densum* C.Agardh was an almost pure fucane (Silchenko et al., 2017a).

6 Conclusion

The marine organisms serve as producers of bioactive compounds in a large amount and diversity presenting elements and compounds of different chemical classes (Barzkar et al., 2017; Barzkar et al., 2018; Jahromi and Barzkar, 2018a; Jahromi and Barzkar, 2018b; Barzkar, 2020; Barzkar and Sohail, 2020; Barzkar et al., 2021a; Barzkar et al., 2021b; Barzkar et al., 2021c; Barzkar et al., 2022a; Barzkar et al., 2022b). Meanwhile, seaweeds play the fundamental role in oxygen producing in the aquatic media, being prolific sources of bioactive agents (Barzkar et al., 2019). Seaweed polysaccharides such as brown algae fucoidan are of value and it is a negatively charged hygroscopic molecule that is chemically saturated with fucose and sulfated polysaccharides, mainly from the extracellular matrix of brown seaweeds. Fucoidan enzymes are a group of hydrolases that hydrolyze complex fucoidan polymers to produce low molecular weight fucoidan.

To date, only the basic principles of the construction of fucoidan molecules have been established. Chemical studies of these polysaccharides are difficult due to the extreme irregularity of their structure, and therefore a detailed analysis of the structure presents significant difficulties. Therefore, a reliable correlation between the chemical structure and the biological action of fucoidans has not yet been elicited. To solve these problems, it is important to use enzymes with established specificity and mechanism of action that can hydrolyze complex fucoidan

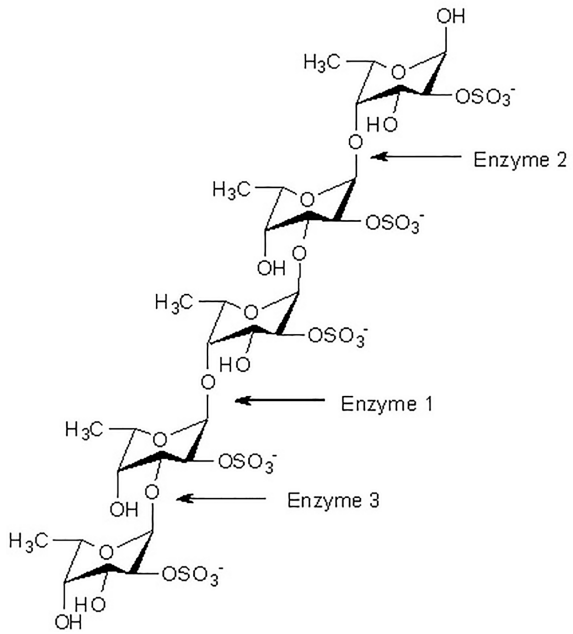

FIGURE 1
Fucoidanase assisted degradation pattern of fucoidan. Enzymatic attack patterns of α-L-fucoidan endohydrolase and fucosidase, respectively: For simplicity, we have distinguished the cleavage patterns as type 1 for (1→4)-α-L-fucoidan endohydrolase and type 2 for (1→3)-α-L-fucoidan endohydrolase; and the exo-action, EC 3.2.1.51, as type 3. (Since the EC 3.2.1.44 fucoidanase activity only designates catalysis of the endo-hydrolysis of (1→2)-α-L-fucosides, the enzymes type 1 and type 2 α-L-fucoidan endohydrolases are in principle not EC categorized.) (Ale and Meyer, 2013).

molecules. A detailed analysis of the structure of small fragments of fucoidans will allow establishing the chemical structure of this polysaccharide. The use of fucoidan fragments of various structures will help to determine the relationship between certain structural elements and their biological action.

Enzymes that use fucoidans as a substrate are called fucoidanases or fucanases. These enzymes act as a hydrolysis catalyst for O-glycosidic bonds within the main chain of fucoidan molecules (EC 3.2.1.44, GH107 (CAZy database)). Fucoidanases are rare and poorly studied enzymes. To date, there is no information on the structural organization of fucoidanases, sizes of active centers, substrate-binding sites, secondary and tertiary structures. Fucoidanases presented a particularly not studied mechanism of action. Such a small number of publications on the structure of fucoidanases and their catalytic properties is due to the low content of these enzymes in organisms used as sources. One solution is to determine the sequences of amino acids inside fucoidanases and obtain recombinant enzymes in sufficient quantities to study specific properties and spatial structure of enzymes (Bakunina et al., 2000).

Fucoidans fermented by fucoidanase can be absorbed into the blood in a small amount and have a direct effect on the circulatory system (Tokita et al., 2010; Nagamine et al., 2014). Nagamine et al. (Nagamine et al., 2014) picturized that low molecular weight fucoidan oligo- and polysaccharides supplied with food are cleaved into small soluble fragments of about 3 kDa and secreted into the extracellular space. Further, these fragments can bind to receptors on the membranes of target cells, mediating effects similar to those during parenteral administration of high molecular weight polysaccharides (Nagamine et al., 2014).

Thus, both direct and indirect biological effects can be expected from fermented fucoidans (Trang et al., 2022). As in the case when fucoidan with low molecular weight fermented by fucoidanase causes a prebiotic effect that can be considered as a direct effect, while the anti-inflammatory effect can be considered as indirect and implemented through an improvement in the composition of the intestinal microflora, an increase in the proportion of symbiotic, and a decrease in the proportion of facultative microflora that has an anti-inflammatory effect (Luthuli et al., 2019; Wang et al., 2019). Fucoidanases can be engaged in the process of sulfated fucooligosaccharides' production (in biotechnology), thus it has potential applications as biologically active additives and drugs.So, the collection of information on this subject indeed need-based attempt or research, and there is an ample scope to explore such enzyme or bioactive compounds for many beneficial purposes, including health, biomedical, bioengineering and life-science research. Hence, the future research strategies may be adopted or designed by the researchers with such latest updated information mentioned in this review article.

Author contributions

Conceptualization and writing original draft, NB. Editing, QL, STJ, VR and RD. Review and funding acquisition, SS, OB All authors contributed to the article and approved the submitted version.

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Conflict of interest

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