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Identification and Bioinformatics Study of Antibacterial Peptides from Symbiotic Bacteria Associated with Macroalgae Sargassum sp

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Abstract

This study aims to identify fragment peptides isolation from symbiotic bacteria associated with macroalgae *Sargassum* sp and predict antibacterial properties from peptides by bioinformatics analysis. Identification fragment peptide using LC-MS/MS with de novo sequencing method. AMP Scanner Vr.2, iAMP-2L, DBAASP used for prediction antibacterial activities. The peptides' potency as antibacterial was evaluated by looking at the peptides' physicochemical properties used APD3 and ProtParam software. The result showed ten peptides sequences, and these peptides were the first reported; five peptides have antibacterial activities according to data from the software. Physicochemical properties from five peptides show the exciting activity to be used as antibacterial agents. The F4h1k peptide sequence has a higher potential according to data physicochemical. However, it is not yet certain which peptides provide the most optimal activity. It needs to be analyzed further.

Keywords: antibacterial, bioinformatic study, de novo sequencing, symbiotic bacteria, peptide.

1. Introduction

Treatment of infectious diseases continues to increase. Treatment of infectious diseases can treat with antibiotics. However, the antibiotic resistance mechanism continues to experience an increase in cases globally [1]. The world urgently needs to change the way it uses antibiotics [2]. Alternative treatment required in order to overcome this problem. Antimicrobial peptides (AMPs) offer an alternative to conventional drugs [3]. AMPs are the important components that can resist the invasion of foreign microor¬ganisms and have broader spectrum antibacte¬rial properties compared to the traditional anti¬biotics [4]

On the way, the peptides produced cannot be ascertained that they can function as antibacterial. Generally, to ensure antibacterial peptides activity through in vivo and in vitro tests. However, along with advances in computational techniques in vitro and in vivo tests, analysis is often preceded by the bioinformatic study as an effective and efficient effort to minimize costs, time, and test use samples [5]. Antimicrobial peptides are highly dependent on amino acid composition, structure, and physicochemical properties [6]. An effective peptide amino acid composition is thought to have a long sequence range of 5 to 25 amino acids with a molecular weight (MW) of less than 10 kDa [7].

There are still not yet many investigations related to peptides from macroalgae as antimicrobial. A total of 42,213 protein sequences from five macroalgae were processed by classifiers that identified 24 possible AMPs by computational tools. If confirmed by *in vitro* extraction and purification techniques,

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these AMPs will first be identified in macroalgae [8]. One literature showed antibacterial activity of a peptide from *Saccharina longicruris*, and from the <10 kDa and >10 kDa fractions was measured and gave significant activity [8]. Leucine-rich repeat sequences have been found in antibacterial peptides originating from this macroalga hydrolysate and are known to increase the antimicrobial potency of AMPs [9].

In recent days, peptide prediction tools will continue to develop and are widely available with easy and fast access. Therefore, this study leads to the search for antimicrobial peptides by the bioinformatics study on the peptides identified by LC-MS/MS with the de novo sequencing method.

2. Methods

2.1. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis

The peptide fragments were obtained from the symbiotic bacteria associated with brown algae, *Sargassum* sp, (code: F4h1) [10]; briefly, the peptide fragments were obtained by enzymatic hydrolysis using pepsin enzyme. LC-MS analysis was performed using the UPLC-MS (ACQUITY UPLC® H-Class System (waters, USA)) equipped with a binary pump following procedure by [11,12].

2.2. Bioinformatic study for AMPs prediction

AMP Scanner Vr.2, iAMP-2L, DBAASP were used to predict antibacterial activity [13]. ProtParam and APD3 analyzed physicochemical properties (amphipathic, charge, aliphatic index, and structure) [14]. Bioactive peptides were modelled with PEP-FOLD3 online software [15].

3. Result and Discussion

3.1. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis

The peptide fragment (code F4h1) were analyzed using the LC-MS/MS instrument. Analysis of LC-MS/MS data using MassLynx software (version 4.1). The results obtained from this software are in the form of a chromatogram from Liquid Chromatography. The m/z spectrum from Mass Spectrometer. The peak chromatogram of F4h1 shows in Fig. 1. In the F4h1 chromatogram there are 13 peaks at the retention time of 2.02; 3.54; 4.08; 4.19; 4,55; 5.09; 5.67; 6.14; 6.54; 6.88; 7,17; 7.44 and 8.29 minutes.

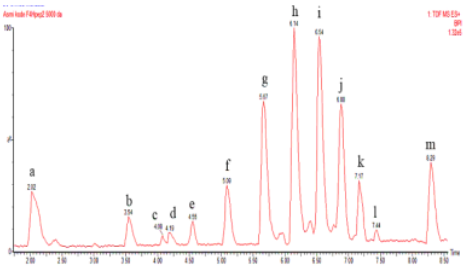

Fig. 1. Liquid chromatography (LC). Chromatogram of F4h1a- F4h1m peptide fraction: (a) F4h1a, (b) F4h1b, (c) F4h1c, (d) F4h1d, (e) F4h1e, (f) F4h1f, (g) F4h1g, (h) F4h1h, (i) F4h1i, (j) F4h1j, (k) F4h1k, (l) F4h1l, and (m) F4h1m.

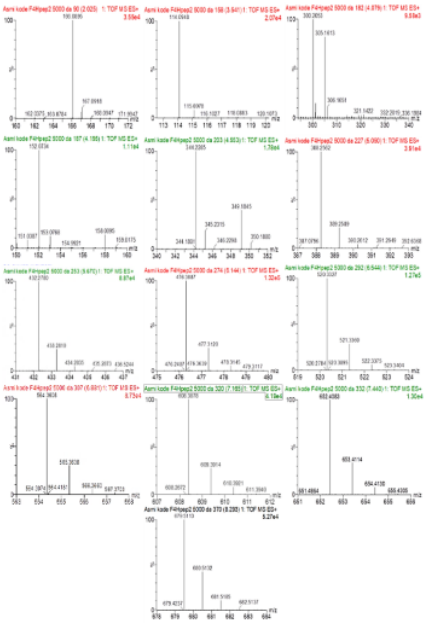

Fig. 2. MS/MS spectra of F4h1a- F4h1m peptide frction: (a) F4h1a, (b) F4h1b, (c) F4h1c, (d) F4h1d, (e) F4h1e, (f) F4h1f, (g) F4h1g, (h) F4h1h, (i) F4h1i, (j) F4h1j, (k) F4h1k, (l) F4h1l, and (m) F4h1m.

The peak at each retention time indicates a different compound [16], so that in this study, the F4h1 peptide fragment assumed to have 13 different types of peptides. The chromatogram of each peak was further analyzed using MassLynx software (version 4.1) to obtain the m/z spectrum value. The

m/z spectrum value for the peptide F4h1 shows in Fig. 2. Peptide identification was made using de novo sequencing (MASCOT mass fingerprint peptide) with NCBI prot as a protein sequence database (Table 1). All peptides fragments come from bacteria-derived protein. According to [7], an effective peptide amino acid composition is thought to have a long sequence range of 5 to 25 amino acids with a molecular weight of less than 10 kDa. Peptides F4h1a, F4h1b, and F4h1d do not qualify for this, so only ten peptides can be analyzed further. Search results on the NCBIprot and UniProt/SWISS-PROT protein and peptide databases for peptide sequences. The ten peptides were the first reported sequences.

Table 1. Result of F4h1 peptide identification with MASCOT program

Code	Peptide sequence	MW (Da)	Score (%)	ID Protein (NCBIprot)	Organisms of origin protein
F4h1a	FPG	n.d	29	1736779	*Californiconus californicus*
F4h1b	P	n.d	17	31087	*Pardachirus marmoratus*
F4h1c	ATLAPLSTLSSL	1173.37	31	877550	*Aspergillus sp. MF297-2*
F4h1d	H	n.d	15	7385	*Sarcophaga bullata*
F4h1e	NTLLVLGRLDCL	1329.62	42	6687	*Penaeus monodon*
F4h1f	MQLQQLEELKEL	1501.76	46	243232	*Methanocaldococcus jannaschii*
F4h1g	MGILDWLSNTLKSGSG	1678.92	43	52351	*Brassica rapa subsp. pekinensis*
F4h1h	MKSLSNFLGSSL	1283.51	42	293813	*Agelena orientalis*
F4h1i	NQFLNGMSLKMPF	1526.83	42	315456	*Rickettsia felis URRWXCal2*
F4h1j	LERFTARAFVVLHV	1657.98	39	234826	*Anaplasma marginale str. St. Maries*
F4h1k	SYLDLAFGCVLKPARH	1790.11	40	12165	*Chrysanthemum virus B*
F4h1l	NKYDLRLPGVLHGLITL PKLGEL	2560.08	36	393305	*Yersinia enterocolitica subsp. enterocolitica 8081*
F4h1m	RKHKLVDCAGAFGAEY ELEVVKGF	2667.08	38	4081	*Solanum Lycopersicum*

3.2. *Bioinformatic study for AMPs prediction*

Many studies have been dedicated to the prediction of antimicrobial peptides (AMPs) by bioinformatic methods. Several online software, such as AMP Scanner Vr.2, iAMP-2L, DBAASP. Based on bioinformatics analysis results (Table 2) of ten peptide sequences that predicted antibacterial activity using online software. Five sequences have potential antibacterial (F4h1c, F4h1e, F4h1g, F4h1h, and F4h1k).

Table 2. antibacterial activity prediction of F4h1a-F4h1m peptides fragment

Code	**Amino acid sequences**	**Amino acid length**	**Antibacterial prediction**		
			1	2	3
F4h1a	FPG	3	n.d	n.d	n.d
F4h1b	P	1	n.d	n.d	n.d
F4h1c	**ATLAPLSTLSSL**	**12**	**NAMP**	**AMP**	**NAMP**
F4h1d	H	1	n.d	n.d	n.d
F4h1e	**NTLLVLGRLDCL**	**12**	**NAMP**	**AMP**	**NAMP**
F4h1f	MQLQQLEELKEL	12	NAMP	NAMP	NAMP
F4h1g	**MGILDWLSNTLKSGSG**	**16**	**AMP**	**AMP**	**NAMP**
F4h1h	**MKSLSNFLGSSL**	**12**	**AMP**	**AMP**	**AMP**
F4h1i	NQFLNGMSLKMPF	13	NAMP	NAMP	NAMP
F4h1j	LERFTARAFVVLHV	14	NAMP	NAMP	NAMP
F4h1k	**SYLDLAFGCVLKPARH**	**16**	**AMP**	**NAMP**	**AMP**
F4h1l	NKYDLRLPGVLHGLITLPKLGEL	23	NAMP	NAMP	NAMP
F4h1m	RKHKLVDCAGAFGAEYELEVVKGF	24	NAMP	NAMP	NAMP

Note: Antibacterial Prediction: Prediction 1 = (AMP Scanner Vr.2 (https://www.dveltri.com/ascan/v2/?u=1584490389722), Prediction 2 = iAMP-2L (http: //www.jci-bioinfo.cn / iAMP-2L, Prediction 3 = DBAASP (https://dbaasp.org/prediction); AMP: Antimicrobial peptides, NAMP=Non Antimicrobial Peptides; nd= not detected, bold=potential antimicrobial peptides (AMPs).

A bioactive peptide amino acid composition is thought to have a long sequence range 5 to 25 amino acids and less than 10 kDa of molecular weight [7]. The ten peptide sequences are included in this category. The presence of specific amino acids and dominant at various peptide positions is related to antibacterial activity.

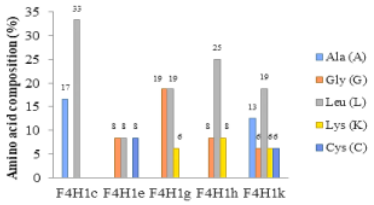

Fig. 3. The percentage of amino acid composition that affects the antibacterial activity of peptides

Amino acids Gly, Ala, Lys, Leu, and Cys, are related to antibacterial activity [17,18]. These amino acids dominant, especially the amino acid Leu, as an identifier for antibacterial activity. The percentage of amino acid composition that affects antibacterial activity can be seen in Fig. 3.

The next factor that affects the antibacterial activity of a peptide is the cationic charge (positive charge) [7,19]. The cationic charge of peptides is the beginning of electrostatic interactions with the anionic charge of bacterial cell membranes [7,17]. The cationic charges value of antibacterial peptide can be seen in Fig. 4 (a); this value is obtained using the online software APD3 and ProtParam. An increase in cationic peptides enhances the antibacterial activity. Although, the positive charges above +9 provides almost no antibacterial activity. Several studies give the data an average charge value of +2 to +9, provide antibacterial activity [19]. The peptide F4h1k has the highest positive charge (+2) compared to four other peptides from the data.

The amphipathic properties of peptides also influence peptides bioactive activity. The amplification level allows the penetration of these peptides through the cancer cell membrane and causes the cancer cell membrane to become unstable [7]. The amphipathic value of peptides shows in Fig. 4 (b). Peptide F4h1k was the highest among the other nine peptide sequences (0.47), followed by peptide F4h1h (0.31). The aliphatic index shows the thermal stability of peptides [20]. Based on Fig. 4(c), the peptide that offers the highest aliphatic index value is the peptide F4h1e (186.67).

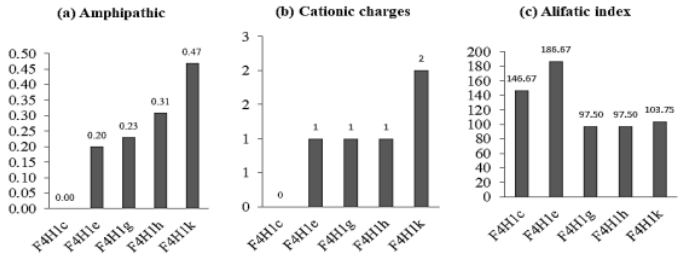

Fig.4. The value of amphipathic (a), cationic charges (b), and aliphatic index (c) of antibacterial peptides

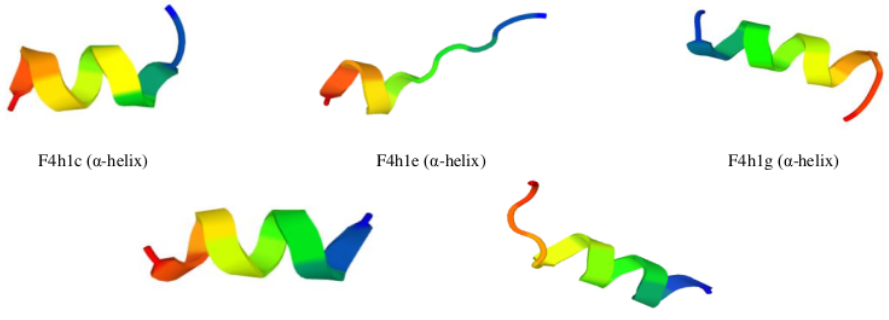

Fig. 5. Prediction of the secondary structure of peptides using PEP-FOLD3 software

The peptide structure also plays a key role in peptide bioactivity. Peptides with an α-helix structure are very promising as antimicrobial therapeutic agents [21,22]. Most α-helical peptides target cell membranes. The interaction between peptides and cell membrane components is a key factor in the selective killing of bacterial and cancer cells. The secondary structure of antibacterial peptides was predicted using online software PEP-FOLD3. The peptide structure prediction results showed that the ten peptides were α-helix structures (Fig. 5). From the data, the amphipathic and cationic charge of F4h1k peptide show the highest potential as an antibacterial peptide so that the peptides can be used for further analysis

4. Conclusions

Bioinformatic analysis is utilized to study the potential of antimicrobial activity from peptides. The identification of peptides from symbiotic bacteria was carried out by Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. From LC-MS/MS data analysis was found ten peptide sequences. These ten peptide sequences are the first reported. Five peptides were active as antimicrobial according to software prediction. The physicochemical of five types of peptides showed potential as antimicrobial. F4h1k peptide has the highest potential as antimicrobial agents. This study provides an overview of the potential peptides as antimicrobial from epiphytic bacteria from macroalgae. Further analysis can be carried out with different bioactivity such as anticancer, antiviral, and others.

5. Conflicts of interest

There are no conflicts to declare.

6. Formatting of funding sources

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