Malaysian Journal of Chemistry, 2022, Vol. 24(2), 47-58
(Special Issue: *2nd IKM Pahang Branch Symposium 2022 – Sustainability & Diversity Through Chemistry*)

Inhibition of *Staphylococcus epidermidis* Biofilm by a Bacteriocin-Like Peptide from *Fejerverya cancrivora*

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Bacterial biofilms are serious threats to human health as they are resistant to both human defence mechanisms and conventional antimicrobial agents. Biofilms can form on surfaces including those of medical devices and cause chronic infections that are difficult to treat, often requiring the use of large doses of antibiotics or the removal of the contaminated device. Anti-microbial peptides (AMPs) isolated from the skin secretion of frogs have been documented as promising anti-biofilm agents. Malaysia is rich in natural resources, including its rainforests, which are a habitat for amphibians. Hence, this study aimed to screen the mucus of the local frog *Fejerverya cancrivora* for potential antibiofilm agents against the biofilm former *Staphylococcus epidermidis* ATCC 35984. The antibiofilm activity of the mucus of *F. cancrivora* was recorded at the attachment, maturation and dispersion stages of biofilm formation, and found to be 97.67 %, 54.66 % and 11.21 %, respectively. The active antibiofilm component was then fractionated and further purified by C18 reverse phase high-performance liquid chromatography (HPLC) to single out peptides with antibiofilm activity. Peptide sequencing revealed a partial amino acid sequence with 67% similarity to an N-terminal bacterial protein belonging to the bacteriocin family. These results suggest that local frog species could be a potential source of antibiofilm peptides.

Key words: Antibiofilm; anti-microbial peptides; *Fejerverya cancrivora*; frog's mucus

Received: December 2021; Accepted: February 2022

A major challenge in modern medicine is the reduced efficacy of antibiotics due to the rapid spread of multi-drug resistant bacteria through indiscriminate and improper use of antibiotics [1-3]. The challenge is intensified by the ability of some bacteria to form biofilm structures with enhanced resistance to antibiotics. In natural environments, most bacteria grow in the form of biofilms and are rarely found in planktonic form [4]. It is estimated that only 0.1% of the total microbial biomass is actually in the planktonic mode of growth while the rest are in biofilm mode [5].

A biofilm is defined as a community of bacteria or microorganisms adhering to a biotic or abiotic surface protected by an extracellular matrix [6]. In bacteria, the formation of a biofilm is believed to occur in stages which include initial attachment of the bacterial cell to a surface, followed by microcolony formation and biofilm maturation in the second stage, while during the final dispersion stage, the bacterial cells detach themselves from the biofilm to find a new surface for adherence [6-7].

When bacteria grow in the form of a biofilm, not only are they able to resist the host's innate and adaptive immune defence mechanisms [8-9], but they can also be up to 1,000-fold more resistant against antibiotics compared to their planktonic counterparts [10]. However, most research on bacterial physiology are generally conducted with planktonic bacterial cells, including testing of the effectiveness of current antibiotics or antimicrobial agents [11]. Hence, while these antibiotics are effective against planktonic cells, they may fail to treat persistent infections caused by bacteria in a biofilm state [12]. Many chronic infections are associated with biofilms. The data shows that more than 80% of bacterial infections are caused by organisms growing in biofilms, particularly in medical apparatus such as cardiovascular devices, catheters and prosthetic valves [13-14]. With the increased use of medical devices in modern medicine, biofilm-associated infections have emerged as a major problem in clinical settings [12].

Hence, the search for new antibiofilm agents has intensified to fight chronic biofilm-associated infections. An antibiofilm agent is a synthetic or natural substance that can facilitate the detachment of mature biofilms or inhibit and/or eliminate biofilm formation *in vivo* [15]. Antibiofilm agents can be divided into three major groups, which include

†Paper presented at the 2nd IKM Pahang Branch Symposium 2022 – Sustainability & Diversity Through Chemistry

peptides, proteins (which are mostly enzymes) and non-peptides [16-18]. In addition, many anti-microbial peptides (AMPs) or host defence peptides isolated from various organisms have shown promising antibiofilm activity [19-20].

AMPs are small molecules consisting of 10 to 100 amino acids with potent antimicrobial activity and are usually part of the innate immune system [21-23]. The first group of AMPs was discovered from the skin of the African clawed frog *Xenopus laevis* [24-25], and since then, a number of AMPs have been reported. Several of these AMPs were found to be good candidates for antibiofilm agents as they displayed antibiofilm activity at different stages of biofilm formation, by decreasing the attachment of bacterial cells and affecting the quorum sensing system, hence downregulating the genes essential for biofilm formation [26-28]. The unique mode of action of both natural and synthetic AMPs targeting multiple sites help to minimise the chances of developing microbial resistance, thus showing potential as the next generation of antibiotics [23].

The most abundant natural source of AMPs comes from frog mucus [29-30]. Frogs are amphibians in the order Anura. They have mucus and granular glands that can produce mucus and serous fluid, respectively, when they are stressed by exposure to environmental conditions [31-32]. The serous fluid contains various bioactive compounds with inhibitory activity toward microbial growth [32-33]. Frogs have thus been widely used in antibiofilm studies due to their ability to produce an abundant range of AMPs [30]. For example, the skin secretion of *Rana chensinesis* exhibited antibiofilm activity against *Streptococcus mutans* biofilms [34], while magainin 2 from *Xenopus laevishas* was found to be active against *Acinetobacter baumannii* biofilms [35]. In Malaysia, 60% of the land is covered by rainforest, one of the main habitats of frogs. There are a total of 254 species of frogs documented in Malaysia, including 111 species in Peninsular Malaysia, 182 species in East Malaysia and 39 species that overlap between Peninsular and East Malaysia [36]. Such abundant resources allow the exploration of the potential of local frogs in producing antibiofilm compounds.

In this study, the antibiofilm potential of the mucus or the skin secretion of *Fejerverya cancrivora* was evaluated. *F. cancrivora* is a species of crab-eating frog found in Southeast Asia, particularly in southern Thailand and Peninsular Malaysia [37-38]. The frog's mucus was tested against the biofilm of *S. epidermidis* ATCC 35984 at the attachment, maturation and dispersion stage, and subsequently, the peptide responsible for the antibiofilm activity was identified.

MATERIAL AND METHODS

Collection of Frogs and Identification

Adult frogs were collected from the Pelangai Forest Reserve, Kuala Pilah, Negeri Sembilan (GPS coordinates: 2.79601, 102.21479). The handling of the frogs was performed under the ethical approval of the Animal Research and Ethics of Universiti Teknologi MARA (UiTM Care: 110/2015).

The collection of slough-off skin cells from the frogs was carried out according to Mendoza et al. (2012) [39]. Each frog was first washed with sterile distilled water, and a sterile cotton bud was rubbed firmly on the frog's body to obtain their skin cells. The genomic DNA was extracted using the DNeasy® Blood and Tissue Kit (Qiagen, Germany) while amplification of 16S rRNA genes was performed using universal primers of the 16S rRNA gene [40] with a forward and reverse primer of 16SA-L (5'-CG CCTGTTTATCAAAAACAT-3') and 16SB-H (5'CC GGTCTGAACTCAGATCACGT-3') respectively. The PCR reaction was carried out using GoTaq® DNA Polymerase (Promega) [41], and the products were visualized on 1.2% agarose gel stained with GelRed (Biotium). DNA sequencing was performed by Eurogentec AITbiotech (Singapore) using the forward primer.

Collection of Frog Mucus

The mucus of the frogs was collected as described in previous studies with slight modifications [41]. The frogs were placed inside a covered container containing absorbent cotton immersed in 1 mL of anhydrous diethyl ether for 1-2 minutes. This would irritate the frogs' skin and result in the secretion of mucus at their dorsal body region while at the same time anaesthetizing the frog [41-42]. The dorsal body region of the frog was then washed twice with 50 mL of 0.1 M sterile saline solution containing 1 % (v/v) of protease inhibitor cocktail (Sigma-Aldrich, USA) to prevent degradation of the peptide molecules. The final solution collected (about 100 mL) was centrifuged to remove debris, and the supernatant was lyophilized and stored at -20 °C. The lyophilized mucus was re-solubilised in 0.1 M pH 6.0 sodium phosphate buffer (PB) to a final concentration of 1 mg/mL [43]. The solution was filter-sterilized using nylon membrane filters with a pore size of 0.22 μm and stored frozen at -20 °C until further use.

Biofilm Inhibition Assay

Biofilm inhibition assays were conducted at the attachment, maturation and dispersion stages as previously described [44-45], with modifications. The biofilm-forming *S. epidermidis* ATCC35984 was used as the test organism (positive control), while a non-biofilm forming strain, *S. epidermidis* ATCC12228 was included as the negative control. Bacterial cultures were prepared by inoculating Tryptase Soy Broth supplemented with 1% glucose (TSBglu) and incubating overnight at 37 °C. These were then diluted in fresh TSBglu at 1:100 and further grown until the

mid-log phase. The turbidity of the cultures was adjusted to 0.08 - 0.13 at OD_{600}, which is equivalent to a cell density of 1 x 10^8 CFU/mL [45-46].

For the biofilm inhibition assay at the attachment stage, 100 µL of the *S. epidermidis* ATCC35984 culture was dispensed into the wells of a flat bottom polystyrene microtiter plate. A volume of 100 µL of the frog mucus was mixed with the bacteria cultures except in the control wells, where 100 µL of PB was added instead. The plate was incubated at 37 °C for four hours to allow the attachment of bacterial cells on the well surface for biofilm formation [45]. The content was discarded, and the plate was carefully washed with 300 µL of PBS and fixed with 150 µL of methanol for 20 minutes. The methanol was then discarded, and the microtiter plate was left inverted to air dry. Following that, the adherent biofilm layer was stained with 150 µL of 1 % crystal violet for 15 minutes, and the excess stain was removed by gentle washing under tap water. The biofilm was air-dried, and the adsorbed crystal violet stain was resolubilized in 150 µL of ethanol for 30 minutes before the optical density was measured at 570 nm.

The biofilm maturation inhibition assay was performed by adding 100 µL of the *S. epidermidis* ATCC35984 culture to the microtiter plate wells and incubating at 37 °C for one hour to allow the attachment of cells on the well surface and initiate the formation of a biofilm [47]. Subsequently, 100 µL of the frog mucus was added to the wells, except for the controls, and then the plate was further incubated at 37 °C for 24 hours. The amount of biofilm formed was then measured as described above.

For the dispersion assay, 100 µL of *S. epidermidis* ATCC35984 culture was dispensed into the microtiter plate wells and incubated at 37 °C for 24 hours to allow biofilm formation and maturation [45]. Next, 100 µL of the frog mucus solution was added to the wells, and incubation was continued at 37 °C for another 24 hours. Subsequently, the residual biofilm material was evaluated in a similar manner as described earlier.

The amount of biofilm formed by the control strains *S. epidermidis* ATCC35984 and *S. epidermidis* ATCC12228 were taken to represent 100 % and 0 % biofilm formation, respectively. The percentage of antibiofilm activity was then evaluated using the following calculation.

The percentage of antibiofilm activity = 100 – ([(S – N) / (P – N)] X 100%)

where:

S = OD_{570} of *S. epidermidis* ATCC35984 + frog skin solution (test samples)

P = OD_{570} of *S. epidermidis* ATCC35984 + 0.1 M PB (positive control)

N = OD_{570} of *S. epidermidis* ATCC12228 + 0.1 M PB (negative control)

The antibiofilm activity was further interpreted as proposed by Famuyide [48] with some modifications. Antibiofilm activity between 1 - 50 % was considered low activity, 51 - 80 % was recorded as high, while a value above 80% was considered very high.

Peptide Purification and Sequencing

0.5 g of lyophilized crude frog mucus was dissolved in 1 mL of 0.1 M PB and filtered through a 0.22 µm nylon membrane filter. The filtrate was applied to a Sephadex G-50 filtration column (diameter 2.6 cm, length 100 cm) equilibrated in PB. Fraction samples were collected at a flow rate of 0.3 mL/min and monitored with a UV detector at 280 nm.

Fractions with high OD_{280} readings were lyophilized, re-dissolved in 2 mL of PB and retested for antibiofilm activity. Fractions with positive results were pooled, lyophilized and re-dissolved in 1 mL of PB. The pooled sample was then applied to a C18 reverse phase high-performance liquid chromatography (RP-HPLC, AGILENT 770995-902 300 Extend-C18, 4.6 x 250 mm, 5 microns) column with 35% trifluoroacetic acid (TFA) and 65 % acetonitrile (ACN), at a flow rate of 1.0 mL/min and wavelength detection at 295 nm. Potential peptide peaks were collected, lyophilised and sequenced using the Applied Biosystems 494 Procise® Protein Sequencing System.

RESULTS

Identification of Frog Species

The results for the amplification of the partial 16S rRNA gene from the frogs' skin are as shown in Figure 1 with the expected amplicon size of approximately 550 bp. The DNA sequences of the partial 16S RNA gene were used to search the nucleotide database at Genbank using the BLAST algorithm. The frog was identified as *Fejervarya cancrivora* with a match of more than 99.5 %, as shown in Figure 2.

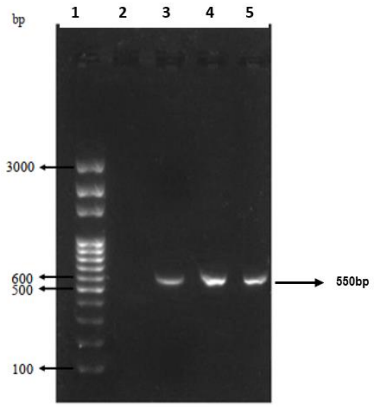

Figure 1. Amplification of the 16S rRNA gene from frog samples
Lane 1: 100 bp DNA ladder; lane 2: negative control; lane 3, 4 & 5: partial 16S rRNA gene of frog samples

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Fejervarya cancrivora haplotype h11 16S ribosomal RNA gene, p...	Fejervary...	1053	1053	100%	0.0	100.00%	570	KX055950.1
☑	Fejervarya cancrivora haplotype h04 16S ribosomal RNA gene, p...	Fejervary...	1048	1048	100%	0.0	99.82%	570	KX055943.1
☑	Fejervarya cancrivora haplotype h08 16S ribosomal RNA gene, p...	Fejervary...	1046	1046	100%	0.0	99.82%	569	KX055947.1
☑	Fejervarya cancrivora haplotype h16 16S ribosomal RNA gene, p...	Fejervary...	1042	1042	100%	0.0	99.65%	570	KX055955.1
☑	Fejervarya cancrivora haplotype h15 16S ribosomal RNA gene, p...	Fejervary...	1042	1042	100%	0.0	99.65%	570	KX055954.1
☑	Fejervarya cancrivora haplotype h10 16S ribosomal RNA gene, p...	Fejervary...	1042	1042	100%	0.0	99.65%	570	KX055949.1
☑	Fejervarya cancrivora haplotype h07 16S ribosomal RNA gene, p...	Fejervary...	1042	1042	100%	0.0	99.65%	570	KX055946.1
☑	Fejervarya cancrivora haplotype h02 16S ribosomal RNA gene, p...	Fejervary...	1042	1042	100%	0.0	99.65%	570	KX055941.1
☑	Fejervarya cancrivora voucher USNM:580908 16S ribosomal RN...	Fejervary...	1042	1042	100%	0.0	99.65%	586	KR816729.1
☑	Fejervarya cancrivora voucher USNM:580900 16S ribosomal RN...	Fejervary...	1042	1042	100%	0.0	99.65%	572	KR816727.1
☑	Fejervarya cancrivora voucher USNM:580872 16S ribosomal RN...	Fejervary...	1042	1042	100%	0.0	99.65%	576	KR816724.1
☑	Fejervarya cancrivora voucher USNM:580845 16S ribosomal RN...	Fejervary...	1042	1042	100%	0.0	99.65%	573	KR816722.1

Figure 2. BLAST Identity Result for *Fejervarya cancrivora*

Inhibition of *S. epidermidis* Biofilm

Figure 3 shows the inhibition results for the mucus of *F. cancrivora* against biofilms produced by *S. epidermidis* on a microtiter plate at the attachment, maturation and dispersion stage. The results of the antibiofilm activity of the frog mucus are shown in Table 1. At the attachment stage, the mucus displayed very high activity with 97.67 % inhibition of biofilm formation. While at the maturation stage, high activity was observed with a 54.66 % reduction of biofilm formation. However, the mucus was less effective at the dispersion stage and only resulted in decreasing the *S. epidermidis* ATCC35984 biofilm by 11.17 %.

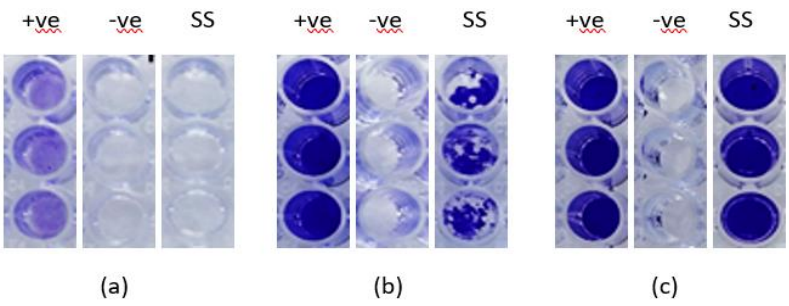

Figure 3. Results of biofilm inhibition at the (a) attachment stage, (b) maturation and (c) dispersion stage.
A microtitre plate showing antibiofilm activity of the mucus of *F. cancrivora* (SS) at the attachment, maturation and dispersion stage together with the positive (+ve) and negative controls (-ve) of biofilm former *S. epidermidis* ATCC35984 and non-biofilm former *S. epidermidis* ATCC12228, respectively.

Table 1. Analysis of antibiofilm activity of *F. cancrivora* mucus against *S. epidermidis* ATCC 35984 at different stages of biofilm formation

Biofilm Stage	Mean OD	Biofilm Formation (%)	Antibiofilm Activity (%)	Strength*
Attachment				
Skin secretion	0.34 ± 0.01	2.33	97.67	+++
Positive control	1.18 ± 0.02	100.00		
Negative control	0.32 ± 0.01	0.00		
Maturation				
Skin secretion	1.37 ± 0.06	45.34	54.66	++
Positive control	2.66 ± 0.02	100.00		
Negative control	0.30 ± 0.01	0.00		
Dispersion				
Skin secretion	3.23 ± 0.02	88.79	11.21	±
Positive control	3.60 ± 0.03	100.00		
Negative control	0.30 ± 0.01	0.00		

*Strength of antibiofilm activity: very high: +++; high: ++; low: +; very low: ±; no activity. Results of mean OD displayed as mean ± S.E.M (standard error of the mean).

Purification and Identification of Antibiofilm Peptide

The mucus of *F. cancrivora* was fractionated using Sephadex G-50 column chromatography and monitored by UV absorbance at 280 nm to isolate the active components [49]. A total of 120 fractions were collected, and fractions with high UV absorbance were pooled into 13 groups labelled F1 - F13, as shown in Figure 4. Each group was then re-assayed for antibiofilm activity at the attachment stage, and the results are shown in Table 2. Significant antibiofilm activity was only observed in fraction F11, which showed 96.78 % inhibition activity at the attachment stage. This activity is similar to that observed in the crude mucus sample, while only low levels of antibiofilm activity were observed in the other fractions. Hence, fraction F11 was most likely to contain the active biomolecules responsible for the observed antibiofilm activity against *S. epidermidis* ATCC 35984.

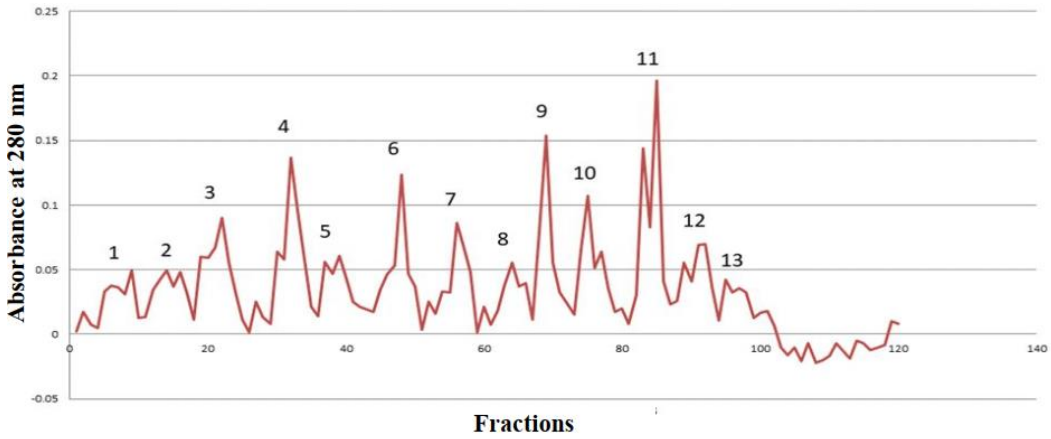

Figure 4. Fractionation of *F. cancrivora* mucus secretion.

Table 2. Analysis of Antibiofilm Activity in fractionated mucus of *F. cancrivora*

Fraction	Mean OD	Biofilm Formation (%)	Antibiofilm Activity (%)	Antibiofilm Strength*
F1	1.14 ± 0.03	95.11	4.89	±
F2	1.17 ± 0.04	98.05	1.95	±
F3	1.14 ± 0.02	95.66	4.34	±
F4	1.13 ± 0.02	93.99	6.01	±
F5	1.17 ± 0.04	98.28	1.72	±
F6	1.18 ± 0.05	99.68	0.32	±
F7	1.13 ± 0.03	94.07	5.93	±
F8	1.18 ± 0.01	99.21	0.79	±
F9	1.16 ± 0.06	97.42	2.58	±
F10	1.13 ± 0.02	94.40	5.60	±
F11	0.34 ± 0.02	3.22	96.78	+++
F12	1.13 ± 0.02	94.08	5.92	±
F13	1.13 ± 0.03	93.87	6.13	±
Positive control	1.18 ± 0.02	100.00	(-)	(-)
Negative control	0.31 ± 0.01	0.00	(-)	(-)

*Strength of antibiofilm activity: very high: +++; high: ++; low: +; very low: ±; no activity. Results of mean OD displayed as mean ± S.E.M (standard error of the mean).

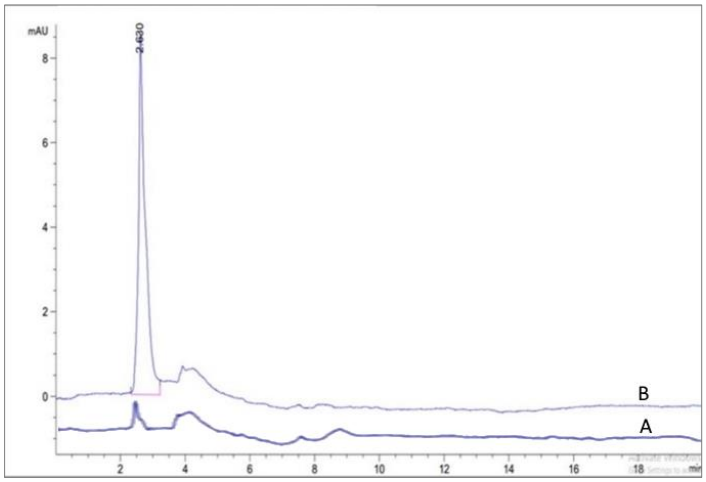

Figure 5. RP-HPLC chromatogram of blank (0.1 M PB) (A) and fraction F11 (B).

HPLC Purification

The active antibiofilm substance in F11 was further purified using reverse-phase HPLC. Figure 5 shows the chromatograms of the blank solution (0.1 M PB) and the antibiofilm compound in F11. A sharp peak with a retention time of 2.630 min was observed in the chromatogram of F11, indicating only one peptide was present; hence no further purification was performed.

Structural Characterization

Peptide end sequencing of the lyophilised sample from Fraction 11 revealed a peptide with the partial N-terminal amino acid sequence of AAPNGLYFGG. This partial sequence was used to search the NCBI protein database (www.ncbi.nlm.nih.gov/protein/), UniPROT (www.uniprot..org) and several AMP databases, i.e. DRAMP (Data repository of antimicrobial peptides, dramp.cpu-bioinfor.org), CAMP (Collection of Anti-Microbial Peptides, www.camp.bicnirrh.res.in), SATPdb (Database of structurally annotated therapeutic peptides, crdd.osdd.net/raghava/satpdb/), BaAMPs (Biofilm-active AMPs database, www.baamps.it), and LAMP2 (Database for Linking AMPs, biotechlab.fudan.edu.cn/database/lamp/index.php). A possible match was found in the CAMP database whereby the amino acid

sequence AAPNGLYFGG was found to be 67 % identical to a part of the hypothetical protein M446_0103 from *Methylobacterium* sp 4-46. This protein was predicted to have antimicrobial activity and is categorized in the bacteriocin family of AMPs.

DISCUSSION

AMPs from the skin secretion of frogs have been widely reported as potential antibiofilm substances [30,33,50]. In 2017, it was reported that the peptide phylloseptin-PC (PSN-PC), from the mucus secretion of the South American tree frog *Phyllomedusa camba*, significantly removed biofilm formed by *S. aureus* [51]. Similarly, in 2017, Gao reported that methicillin-resistant *S. aureus* or MRSA biofilm was inhibited by the peptide medusin-PT from the Tarsier Leaf Frog, *Phyllomedusa tarsius* [52]. Another peptide, ranatuerin-2Pb, isolated from the mucus secretion of *Rana pipiens* [53], displayed the ability to inhibit and reduce the biofilms of *S. aureus*, *Escherichia coli* and *Candida albicans*. Similarly, in the present study, a peptide from the crab-eating frog *F. cancrivora* was found to have antibiofilm activity against *S. epidermidis* ATCC 35984. Thus, the presence of antibiofilm activities in frog AMPs appears to be widespread.

However, in most of the studies involving inhibition of antibiofilm by frog mucus, it was observed that the bioactive compounds responsible displayed high antibiofilm activity when they were tested at the early stage of biofilm formation. In contrast, at the later stage of formation, the dispersion stage, the biofilm appears to be more resistant. In 2020, the mucus peptide brevinin-GR23 (B-GR23) was extracted from *Hylarana guentheri*, a dominant frog species in Hainan Island of southern China, and this was found to be able to inhibit biofilm formation of *S. aureus* at all stages [54]. Another report showed that all stages of biofilm formation by *Streptococcus mutans* was inhibited by temporin-GHc (GHc) and temporin-GHd (GHd) peptides from *H. guentheri* [55]. However, in both cases, the highest inhibition activity was observed at the attachment stage, followed by the maturation and dispersion stages. Similarly, in the present study, *F. cancrivora* was found to have antibiofilm activity against *S. epidermidis* ATCC 35984 at the attachment, maturation and dispersion stages with 97.84 %, 54.50 % and 11.17 % inhibition, respectively. Thus, a higher percentage of inhibition was displayed in the early stages of biofilm formation.

The same observation was reported in another study. In 2011, the antibiofilm activity of the short peptide, F(2,5,12)W, was tested against a weak biofilm producer *S. epidermidis* strain BM185, and *S. epidermidis* strain BM492, a strong biofilm producer [56]. It was found that the antibiofilm activity of the peptide was reduced as the biofilms of both strains matured. Staphylococcal biofilm matrix contains polysaccharide intracellular adhesin (PIA), which forms a thick layer at the maturation and dispersion stage as the biofilm develops. PIA is known to be able to reduce the penetration of AMPs into the biofilm structure [12,57]. Hence, this might be another reason for the reduction of antibiofilm activity at the maturation and dispersion stages compared to the attachment stage. In addition, the strength of the biofilm formed might also affect the activity of antibiofilm compounds. The antibiofilm activity of F(2,5,12)W was stronger against *S. epidermidis* BM185 compared to *S. epidermidis* BM492.

The present study revealed that mucus secretion from *F. cancrivora* species might contain a new antibiofilm peptide with an amino acid sequence similar to part of the hypothetical protein M446_0103 from *Methylobacterium* sp. 4-46. Bacteriocins are proteinaceous toxins or antimicrobial peptides (AMP) produced by bacteria [58] and are usually active against bacterial strains closely-related or non-related to producer strains [59-61]. It was reported that 99% of bacteria could produce at least one bacteriocin [62]. Many bacteriocins have been isolated and reported, and some display the ability to kill and inhibit biofilm formation [63-66]. For example, nisin A produced by *Lactococcus lactis* and some *Streptococcus* strains [64,67-68] has antimicrobial and antibiofilm activities and is highly active against various Gram-positive bacteria, including the *Listeria*, *Staphylococcus*, *Bacillus* and *Enterococcus* species [69-73]. Nisin was also reported to inhibit biofilm formation by Gram-negative bacteria, especially oral pathogens such as *Porphyromonas gingivalis*, *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans* and *Treponema denticola* [74-75]. Gallidermin is a bacteriocin produced by *Staphylococcus gallinarum* that exhibits antimicrobial activity against *Propionibacterium acnes* [76]. This bacteriocin can also inhibit the biofilms of *S. aureus* and *S. epidermidis* on implanted medical devices such as stents and catheters [77-79]. Similarly, the bacteriocin epidermin produced by *S. epidermidis* also has antibiofilm activity against *S. epidermidis* and *P. acnes*. Interestingly, the amino acid sequence of epidermin is very similar to gallidermin [76]. In 2012, it was reported that enterocin AS-48 from *E. faecalis* can inhibit *L. monocytogenes* biofilm formation at the concentration of 50 µg/mL. They also reported that using enterocin in combination with biocides enhanced the ability of this bacteriocin to inhibit the biofilm of *L. monocytogenes* [80].

Compound F11 was able to inhibit the biofilm of *S. epidermidis* ATCC35984. Although the partial N-terminal sequence of F11 was similar to the sequence of a bacteriocin from the *Methylobacterium* species, there have been no reports on bacteriocin from this species being used as antibiofilm agents. However, in 2015, Tejesvi *et al.* reported that *Methylobacterium extorquens* produced a defensin-like peptide MB1533, the first AMP reported from the

Methylobacterium genus [81]. Defensin-like peptides displayed a combination of antifouling and antimicrobial properties that might inhibit biofilm formation [82]. Therefore, F11 from *F. cancrivora* could be a new antibiofilm peptide similar to *Methylobacterum* AMPs.

CONCLUSION

A peptide extracted from the mucus secretion of the *F. cancrivora* frog species was found to possess the ability to inhibit bacterial biofilm at all stages. However, the strength of this antibiofilm peptide decreased at the maturation and dispersion stage. Partial amino acid sequencing indicated that this may be a novel antibiofilm peptide. Further studies are needed to fully characterize this peptide and elucidate the mechanisms by which it inhibits biofilm formation. The data from this study is a significant contribution to the search for new AMPs from frogs that could be used as novel antibiofilm agents.

ACKNOWLEDGEMENT

Authors thank the Ministry of Higher Education Malaysia for funding through the Fundamental Research Grant Scheme (FRGS/1/2018/STG05/ UITM/03/3) and the Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM) Shah Alam, Malaysia. Peptide sequencing services were provided by the Australian Proteome Analysis Facility supported under the Australian Government's National Collaborative Research Infrastructure Strategy (NCRIS).

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