Therapeutic Efficacy of Antibacterial Ocellatin Peptides—A Comprehensive Review

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Abstract: Antimicrobial peptides (AMPs), ascribed to their decreased microbial drug resistance, can be employed as potent small-molecule drugs to treat various diseases. AMPs have been conserved in a wide variety of living organisms as a result of the evolution of the innate immune system. Notably, Ocellatin AMPs derived from South American Leptodactylus genus frogs have a higher therapeutic efficacy against infections. Inhibitory activity of Ocellatin AMPs against bacterial membranes is determined by the dynamic interplay of peptide cationic, hydrophobicity, helicity, and amphipathicity. Another advantage of using AMPs as drug candidates is their cell selectivity that is non-hemolytic to human cells. Ocellatin AMPs with optimal hydrophobic residues would therefore be a recommended therapeutic candidate. Henceforth, such AMPs could be used as an alternative strategy in curbing antimicrobial resistance. It is noteworthy that the therapeutic efficacy of Ocellatins is to be appreciated for its broad application as it has been proved to be active against several humans, animal, and plant bacterial pathogens.

Keywords: antimicrobial peptides; Ocellatin, antibiotic resistance; membranolytic peptides; therapeutic modifications.

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1. Introduction

Antibiotics, invented by Alexander Fleming after World War I, have been used as a wonder drug for almost a century [1]. However, such antibiotics are becoming a failure due to their extensive overuse in recent decades, resulting in antimicrobial resistance (AMR) [2]. This alarming rate of antibiotic failure prompted scientists to emphasize alternate therapeutics [3–5]. Nothing in biology makes sense unless viewed from the perspective of evolution, from which we seek asylum in antimicrobial peptides (AMPs), that has evolved as innate host molecules in a wide range of organisms around 2.5 billion years ago [6–8].

Compared to AMPs from other organisms, AMPs isolated from the skin secretion of anurans (frogs and toads) hold out as a promising therapeutic candidate for antimicrobial resistance [9]. Generally, the skin serves as a gateway between an organism’s internal and external environments, as well as a barrier to a variety of microbes attempting to invade our cells as their home [10]. The skin of anurans, in particular, has evolved AMPs to enable them to survive and thrive in both terrestrial and aquatic habitats [11]. According to Antimicrobial Peptide Database-3 (APD3), over 980 AMPs out of 1093 anuran AMPs demonstrated antibacterial activity [12].
More specifically, experimental studies have established that Ocellatin peptides derived from the skin secretions of *Leptodactylus* genus frogs have a broad spectrum of antibacterial activities [13]. Nascimento et al., 2004 first discovered Ocellatin-1 in the skin dorsal glands of the Amazonian Argus frog, *Leptodactylus ocellatus*, and reported it to be active against the Gram-negative bacterium *E.coli*. Inconsequent studies, the peptides from the same species [14–16] and evolutionarily similar frog species *L. pustulatus* [17], *L. syphax* [18] *L. validus* [19] *L. laticeps* [20], and *L. labirynthicus* [21,22] have revealed the membranolytic activities against several bacteria including multidrug-resistant strains [23]. Moreover, recent studies on *L. latrans* [24], *L. vastus* [25], *L. insularum* [26], and *L. nesiotus* [26] have further revealed antibacterial properties of newly characterized Ocellatin AMPs. Considering the lack of inclusive data regarding this emerging topic, a comprehensive review of the aforementioned Ocellatin peptides in terms of their therapeutic potential is presented.

2. Basic Features of Anuran AMPs and their Action Mechanism

Anuran AMPs are mostly alpha-helical amphipathic peptides containing hydrophobic and hydrophilic residues with a range in length from 5 to 50 amino acids. In general, these AMPs are cationic because they contain more positively charged amino acids such as lysine (K), arginine (R), and histidine (H) [27,28]. The cationic property, frog-skin peptides in their early phase are electrostatically attracted to the anionic bacterial membrane [29]. Following that, such AMPs self-associate as carpet, barrel-stave, or toroid models, as illustrated in Figure 1.

Self-associated AMPs can permeabilize bacterial membranes in two ways either micellization or pore formation [30]. For instance, Aurein AMP from the Golden-bell frog (*Litoria aurea*) develops a detergent-like carpet model after reaching a particular threshold level and aggregates around the membrane, leading to micellization [31]. On the other hand, toroidal and barrel-stave models result in pore formation, leading to membrane depolarization and cell death. For instance, the Magainin AMPs from African clawed frog (*Xenopus laevis*) assemble into transmembrane pores of the toroidal type [32]. Also, the buforin AMPs from Asiatic toads (*Bufo gargarizans*) self-associate in the barrel-stave paradigm, which allows hydrophobic components of peptides to interact with hydrophilic membrane surface [33]. As a result, a transmembrane pore forms with a core lumen, which is followed by an efflux of cellular constituents and consequent cell death.

![Figure 1. General mechanism of action of anuran alpha-helical AMPs against the bacterial membrane.](https://biointerfaceresearch.com/)
3. Selective Permeability of Ocellatin Peptides

In general, AMPs with high bacterial permeability are considered valuable in therapeutics, where the cell permeability is determined by amphipathic (hydrophobic and hydrophilic) residues [34]. Amphipathicity of AMPs ought to be optimal because a greater value will enable them to penetrate eukaryotic membranes, while a lower value will prevent an AMP from even penetrating bacterial membranes [35,36]. However, Ocellatin AMPs with optimum hydrophobic residues would indeed be a preferred therapeutic priority, which has been demonstrated in various investigations. Moreover, the self-associating tendency of AMPs is also dependent on their immense amphipathic values [37]. Similarly, increased amphipathic values found in Ocellatin-4 exhibit a cascade of antibacterial activity as a result of its self-association in solutions.

Another property of AMPs that determines membrane permeability is their cationic [38]. In an experimental evaluation of Ocellatin-L2, it has been revealed that when negatively charged residues in the peptide were replaced with neutral residues, the peptide’s capacity to inhibit E. coli was compromised. Positive charges in peptides have been proven to exhibit enhanced bacterial permeability [39]. On the other hand, cationic Ocellatin AMPs selectively avoid adhering to zwitterionic eukaryotic membranes. AMPs are notably specific towards bacterial cells due to their net charge. For instance, in Ocellatin-4, two lysine residues have been neutralized by two aspartate residues, enabling it to be both antibacterial and non-hemolytic [40]. Conversely, the presence of an extra negatively charged aspartate residue in Ocellatin-PT peptides impedes negatively charged bacterial membrane, resulting in its decreased antibacterial efficacy. In our previous computational analyses, we revealed that Ocellatin-1’s net charge was lower than Ocellatin-F1, Ocellatin-K1, and Ocellatin-S1 due to the presence of negatively charged glutamic acid at position 23 (E23) [40]. Moreover, this conservation of E23 is found consistent with experimental results on Ocellatin-V1, Ocellatin-V2, Ocellatin-V3, and Ocellatin-P1.

4. Antibacterial Efficacy and Biofilm Inhibiting Potency

It is worth noting that numerous Ocellatin AMPs are efficient against a wide variety of bacterium, as denoted in Figure 2 [20]. Several Gram-negative bacteria, including Escherichia coli (E. coli), Klebsiella pneumoniae (K. pneumoniae), and Salmonella choleraesuis (S. choleraesuis) were inhibited by Ocellatin-PT1, Ocellatin-PT2, Ocellatin-PT4 and Ocellatin-PT8 peptides produced from L. pustulatus [24]. Peptides such as Ocellatin-S1 are equally effective to Gram-negative bacteria such as Escherichia coli and Gram-positive bacteria such as Staphylococcus aureus (S. aureus). However, various peptides, including Ocellatin-P1, Ocellatin-L1, and Ocellatin-F1, have been found to be more efficient against Gram-negative than Gram-positive bacteria (Table 1).

| S.No | Peptide name | UNIPROT ID | Species | Sequence | Active against | Reference |
|------|--------------|------------|---------|----------|---------------|-----------|
| 1    | Ocellatin-1  | P83951     | L. ocellatus | GVVDILKGAGKDLLAHVLVGKISEKV | E. coli | [14]       |
| 2    | Ocellatin-2  | P83866     | L. ocellatus | GVLDFKDAAKQILAHAAEOI | E. coli | [14]       |
| 3    | Ocellatin-3  | P83867     | L. ocellatus | GVLDFKDAAKQILAHAAEOI | E. coli | [14]       |
| 4    | Ocellatin-4  | P85090     | L. ocellatus | GLLDFVTGVDIFDIAFQLIKQ | E. coli | [14,15]   |
| 5    | Ocellatin-4 analogue | -NA-     | L. ocellatus | KLLKFVTKVGKAIKALKAI | X. citri | [41]        |
|      |              |            |          |          | S. meliloti |           |

Table 1. List of antibacterial Ocellatin peptides.
| S.No | Peptide name | UNIPROT ID | Species | Sequence | Active against | Reference |
|------|--------------|------------|---------|----------|----------------|-----------|
| 6    | Ocellatin-5  | P85443     | *L. ocellatus* | GLDILGKAAGKVLTVNL | *E. coli* | [14] |
| 7    | Ocellatin-6  | -NA-       | *L. ocellatus* | AVLDFIKAAGKGLTVNIMEKV | *E. coli* | [16] |
| 8    | Ocellatin-7  | -NA-       | *L. latrans* | GVVDILKDTGKLLSLMEKIG | -NA- | [24] |
| 9    | Ocellatin-8  | -NA-       | *L. latrans* | GVVDILKDTGKLLSLMEKIG | -NA- | [24] |
| 10   | Ocellatin-9  | -NA-       | *L. latrans* | GVLDIFIKDTGKLLSLMEKIG | -NA- | [24] |
| 11   | Ocellatin-10 | -NA-       | *L. latrans* | GLDILGKAAGKVLTVNKEVG | -NA- | [24] |
| 12   | Ocellatin-11 | -NA-       | *L. latrans* | GLDIFKDAAGKGLAHAAEKIG | -NA- | [24] |
| 13   | Ocellatin-F1 | P0DQ38     | *L. fallax* | GVVDILGKAADIGHLASKVMNK | *E. coli* | [21,22,43] |
| 14   | Ocellatin- F1(1-22) | C0HKF1 | *L. labrinthicus* | GVVDILGKAADIGHLASKVM | *A. actinomycetemcomitans* | [21,22] |
| 15   | Ocellatin- F1(1-23) | C0HKF2 | *L. labrinthicus* | GVVDILGKAADIGHLASKVM | *A. actinomycetemcomitans* | [21,22] |
| 16   | Ocellatin-K1 | P86711 | *L. knueseni* | GLDILGKAADIGHLASKVMK | -NA- | [25,40] |
| 17   | Ocellatin- K1(1-16) | -NA- | *L. vastus* | GVVDILGKAADGLAGH | -NA- | [25] |
| 18   | Ocellatin- K1(1-21) | -NA- | *L. vastus* | GVVDILGKAADGLHASKV | -NA- | [25] |
| 19   | Ocellatin-1I | -NA-       | *L. insularum* | GLDILGKAADIGHLASKVM | *E. coli* | [26] |
| 20   | Ocellatin-1I(1-16) | -NA- | *L. insularum* | GLDILGKAADIGHLASKVM | *K. pneumonia* | [26] |
| 21   | Ocellatin-2I | -NA-       | *L. insularum* | GLDILGKAADIGHLASKVM | *E. coli* | [26] |
| 22   | Ocellatin-2I(1-16) | -NA- | *L. insularum* | GLDILGKAADIGHLASKVM | *P. aeruginosa* | [26] |
| 23   | Ocellatin-3I | -NA-       | *L. insularum* | GLDILGKAADIGHLASKVM | *K. pneumonia* | [26] |
| 24   | Ocellatin-1N | -NA-       | *L. nesiotus* | GLDILGKAADIGHLASKVM | *E. coli* | [26] |
| 25   | Ocellatin-2N | -NA-       | *L. nesiotus* | GLDILGKAADIGHLASKVM | *P. aeruginosa* | [26] |
| 26   | Ocellatin-3N | -NA-       | *L. nesiotus* | GLDILGKAADIGHLASKVM | *S. typhimurium* | [26] |
| 27   | Ocellatin-4N | -NA-       | *L. nesiotus* | GLDILGKAADIGHLASKVM | *P. aeruginosa* | [26] |
| 28   | Ocellatin-S1 | P85279     | *L. syphax* | GLDILGKAADIGHLASKVM | *E. coli* | [18] |
| 29   | Ocellatin-L1 | P0DQL0     | *L. laticeps* | GLDILGKAADIGHLASKVM | *E. coli* | [20] |
| 30   | Ocellatin-L2 | P0DQL1     | *L. laticeps* | GLDILGKAADIGHLASKVM | *E. coli* | [20] |
| 31   | Ocellatin- PT1 | C0HJZ6 | *L. pustulatus* | MAFKLKSLFLVFLGLVSLICDEEK RQDEDDDDDEEK RVVDIADKAGQ LVHAMAHKIAEKV | *E. coli* | [17] |
| 32   | Ocellatin- PT2 | C0HJZ7 | *L. pustulatus* | MAFKLKSLFLVFLGLVSLICDEEK RQDEDDDDDEEK RVVDIADKAGQ LVHAMAHKIAEKV | *K. pneumonia* | [17] |
| 33   | Ocellatin- PT3 | C0HJZ8 | *L. pustulatus* | MAFKLKSLFLVFLGLVSLICDEEK RQDEDDDDDEEK RVVDIADKAGQ LVHAMAHKIAEKV | *S. aureus* | [17] |
| S.No | Peptide name | UNIPROT ID | Species       | Sequence                                      | Active against                                      | Reference |
|------|--------------|------------|---------------|-----------------------------------------------|------------------------------------------------------|-----------|
| 34   | Ocellatin-PT4 | C0HJZ9     | *L. pustulatus*| MAFLKSLFLVLGLVLSICDEEKRQDEDDDDDEEKEKRGVIKAGQQLIAHAMGKIAEKV | *E.coli* *S.aureus* *K.pneumoniae* *S.choleraesuis* | [17]      |
| 35   | Ocellatin-PT5 | C0HK00     | *L. pustulatus*| MAFLKSLFLVLGLVLSICDEEKRQDEDDDDDEEKEKRGVIKAGQLVAHAMGKIAEKV | *E.coli* *S.aureus* *K.pneumoniae* *S.choleraesuis* | [17]      |
| 36   | Ocellatin-PT6 | C0HK01     | *L. pustulatus*| MAFLKSLFLVLGLVLSICDEEKRQDEDDDDDEEKEKRGVIKAGQLIAHAMEKIAEKVGLNKDGN | *E.coli* *S.aureus* *K.pneumoniae* *S.choleraesuis* | [17]      |
| 37   | Ocellatin-PT7 | C0HK02     | *L. pustulatus*| MAFLKSLFLVLGLVLSICDEEKRQDEDDDDDEEKEKRGVIKAGQLIAHAMEKIAEKVGLNKDGN | *E.coli* *S.aureus* *K.pneumoniae* *S.choleraesuis* | [17]      |
| 38   | Ocellatin-PT8 | C0HK03     | *L. pustulatus*| MAFLKSLFLVLGLVLSICDEEKRQDEDDDDDEEKEKRGVIKAGQLIAHAMEKIAEKVGLNKDGN | *E.coli* *S.aureus* *K.pneumoniae* *S.choleraesuis* | [17]      |
| 39   | Ocellatin-P1  | P0DQ17     | *L. pentadactylus*| GLDTLKGAANVVGSLASKVMEKL | *E.coli* *E. cloacae* *K.pneumoniae* *P.aeruginosa* *S.aureus* *S. epidermidis* *E. faecalis* | [43]      |
| 40   | Ocellatin-V1  | -NA-       | *L. validus*  | GVVDILKGAAGKDLLAHALSKLEK | *E.coli* *S.aureus* | [19]      |
| 41   | Ocellatin-V2  | -NA-       | *L. validus*  | GVLIDLKGAAGKDLLAHALKISEK | *E.coli* *S.aureus* | [19]      |
| 42   | Ocellatin-V3  | -NA-       | *L. validus*  | GVLIDLTGAGKDLLAHALKSEK | *E.coli* *S.aureus* | [19]      |

-NA- Not Applicable

4.1. Gram negative bacteria.

Gram-negative bacteria like *E.coli* and *K. pneumoniae* are common in our gut as commensals and do not affect humans. However, they begin as primary food contamination and expect multi-organ diseases affecting the lungs, brain, heart, and urinary tract. In which nearly all Ocellatin peptides inhibit *E.coli* (Table 1). Conversely, Ocellatin-F1 and Ocellatin-PT1-PT8 excluding PT2 inhibited carbapenem-resistant *K. pneumoniae* bacteria producing lung illness [42, 43]. *Salmonella typhimurium* (*S. typhimurium*), a food-borne pathogen, is resistant to antibiotics such as chloramphenicol, tetracycline, cephalosporins, sulfonamides, and streptomycin [44]. Owing to its multidrug-resistant nature, typhoid and associated gastroenteritis in humans turn challenging to treat [45]. However, Ocellatin 1N, 1I, 2I, 3N exhibited resistance against *S. typhimurium* with minimum inhibition concentration (MIC) at the range of 31.25–62.5 μM.

Another opportunistic Gram-negative bacterium resistant to many drugs is *Pseudomonas aeruginosa* (*P. aeruginosa*) [46]. The evolved communication mechanisms in *P. aeruginosa*, besides quorum sensing, endorsed it to develop biofilms [47,48]. However, AMPs act as a new class of anti-biofilm compounds since they can either restrict biofilm formation or remove established biofilms [49,50]. *P. aeruginosa* can be inhibited with Ocellatin PT3 (MIC=16 μg/ml), which is the most effective of the extensively investigated Ocellatin peptides (PT2-PT6) [51].
Figure 2. A schematic representation depicting Ocellatin peptides and three major forms of diseases they combat.

*S. choleraesuis* is yet another Gram-negative bacterium that causes swine pneumonia in pigs and affects the pork supply chain [52]. Moreover, the contaminated pork poses a health risk to humans. Because it causes illness in pigs and humans, the economic cost is doubled by *S. choleraesuis* [53]. Ocellatin-PT1 and PT3-PT8 may mitigate Salmonella infection, whereas Ocellatin-PT2 binds to lipopolysaccharide (LPS) at first, but the peptide cannot suppress Gram-negative bacterial growth [51,54]. *Xanthomonas citri* (*X. citri*), a common phytopathogen, is a rod-shaped Gram-negative bacterium [55,56]. Interestingly, analogs of Ocellatin-4 have been proven to be potent against *X. citri*, preventing citrus fruit damage and possibly increasing the economic value. *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) is another Gram-negative facultative bacterium that can cause aggressive dental gum inflammation (periodontitis) [57]. Periodontitis may be caused by non-infectious risk factors like smoking, stress, and aging, and hormonal changes in addition to bacterial infection that result in dental biofilms [58,59]. However, Ocellatin-F1 (1-22) and Ocellatin-F1 (1-23), commonly known as Ocellatin-LB1 and Ocellatin-LB2, are employed to treat periodontitis.

4.2. Gram-positive bacteria.

The gram-positive bacterium, *Staphylococcus aureus* (*S. aureus*), causing multi-organ illness, is still a serious public health problem, as it causes nosocomial (hospital-acquired) infections [60]. Ocellatin-3N, Ocellatin-5, Ocellatin-6, Ocellatin-F1, Ocellatin-S1, Ocellatin-L1, and Ocellatin-PT1-PT8, all curbed methicillin-resistant *S. aureus*. *Enterococcus faecium*, a Gram-positive gut commensal, causes a significant proportion of hospital-acquired infections with high mortality [61]. Ocellatin-3N, on the other hand, acts potently against that bacteria.
5. Therapeutic Modifications to Enhance Antibacterial Potential

One of the essential post-translational modifications in any alpha-helical AMP is C-terminal amidation in which the hydroxyl (OH) group is substituted with the amide (NH2) group at C-terminus [62,63]. C-terminal amidation regulates peptide stability and functionality in various ways viz., i) stabilizing helicity ii) increasing cationic iii) altering dipole moment iv) degenerating carboxypeptidases and thereby, (v) increasing bactericidal activity [64]. C-terminal amidation aspects have been reported to be highly effective in Ocellatin peptides such as Ocellatin-PT1 to Ocellatin-PT5, Ocellatin-5, and Ocellatin-6 (Figure 3).

![Figure 3. The illustration of therapeutic modifications in Ocellatin peptides notably, C-terminal amidation and antibacterial synergism.](image)

Synergizing the effects of antibiotics with AMPs is beneficial for combating multidrug-resistant bacteria [65]. Cefazidime (cephalosporin antibiotic) and Ciprofloxine (quinolone antibiotic) have been synergized with Ocellatin-PT3. With the help of Ocellatin-PT3, these alkaloid drugs have substantially improved their permeability of cell membranes of multi-drug resistant (MDR) isolates. Moreover, for evaluating antiviral activity against the Rabies virus, Ocellatin-I was conjugated with the serotonin-derived alkaloid Bufotenine [66].

6. Limitations and Future Perspectives

There are a few drawbacks of using AMPs on a wide scale. Mostly alpha-helical AMPs are degraded by extracellular proteinases due to their co-evolution with AMP-producing hosts. Henceforth, the pathogens also develop various resistance mechanisms such as the loss of activity at human physiological salt concentrations and increased hemolytic effect cleaving human erythrocytes. Another important limitation in using AMP like Ocellatin is the lack of appropriate strategies to deliver drugs sustainably [67].

Pertinently, this could be avoided in the future by incorporating D-form non-natural amino acids or by employing non-peptide backbones via peptidomimetics, which could lead to the development of alpha-helical AMPs as potential antimicrobial drugs. Particularly, D-amino substitutions will reduce the hemolytic activity by increasing the antibacterial activity simultaneously [68,69]. Further, understanding the supramolecular chemistry of peptide self-assembly can better understand how biofilms and secondary targets function, leading to new directions in the formulation of self-assembled Ocellatin peptide nanostructures in therapeutics. Furthermore, the emergence of new big data design methods like machine
learning will facilitate researchers to explore new targets for Ocellatin AMPs other than the membrane.

7. Conclusions

To summarise, the entire globe seeks novel drugs to combat alarming antimicrobial resistance and antibiotic failure. However, the alpha-helical AMPs isolated from multi-habitat organisms such as frogs would be a preferable alternative for the pharmaceutical industry to employ AMPs as medications in the future. Considering the versatility of Ocellatin AMPs, they can be used in animal husbandry and agricultural sectors in addition to clinical applications. We anticipate that this review article will help pharmaceutical and agricultural researchers to understand the potential of Ocellatin.

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Conflicts of Interest

The authors declare no conflict of interest.

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