Review Paper:

Black soldier fly (BSF): a source of antimicrobial peptides

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Abstract

Hermetia illucens, commonly known as Black Soldier Fly (BSF) survives on waste decaying matter in presence of abundant pathogenic bacteria. It metamorphoses in stages like egg, larva, pupa and adult. The BSF sustains in optimal space, temperature, light and humidity. Since it resides admist the pathogenic environment, it features variety of antimicrobial peptides including defensins, cecropins, attacins, diptericins etc. Different mechanisms used by these AMP's to target bacterial membrane include toroidal pore model, carpet like model and barrel stave model whereas non membrane targeting mechanisms include inhibition of protein biosynthesis, nucleic acid biosynthesis, protease activity and cell division.

The activity of the AMP depends on the environmental factors like metal ions, pH, proteases, salt concentrations and temperature. These peptides have ability to kill various pathogenic microbes like Staphylococcus spp., Streptococcus spp., E. coli, Candida albicans etc. These AMP's are extracted by the processes of immunization of larvae followed by extraction of AMPs. The extracted peptides are then purified and stored. The AMPs from BSF will have potential applications across various fields of medicine, agriculture and food industries due to their broad-spectrum antimicrobial properties.

Keywords: BSF, Antimicrobial peptides, Peptide Extraction, Peptide Purification.

Introduction

The Black Soldier Fly (BSF), *Hermetia illucens* (Diptera: Stratiomyidae), is a thermophilic, harmless insect renowned for its efficiency in breaking down organic waste like food scraps, straw and manure. BSF larvae surpass other insects, such as *Drosophila melanogaster* and *Apis mellifera*, in their consumption rates of these substrates[45]. Taxonomically, BSF belongs to the Stratiomyidae family and exhibits wasp-like mimicry with elongated antennae and transparent abdominal "windows". Native to the Western hemisphere, this species thrives in decomposing environments, particularly in the southeastern United States, where it has three annual generations. However, its mass production faces threats from predators like the parasitoid wasps *Dirhinus giffardii* and *Eniacomorpha hermetiae*[22]. Recognizable by their gray-black striped larvae, BSFs are widely used to manage animal waste in swine and poultry farms[80].

Although adult flies do not sting or spread diseases, they may act as mechanical vectors for pathogens, with accidental ingestion posing minimal risks[28].

Life Cycle of the Black Soldier Fly

The entire life cycle of the black soldier fly can vary in duration depending on temperature and environmental conditions, but typically ranges from 25 to 42 days from egg to adult emergence. It is characterized by several distinct stages from egg, being the youngest stage and adult fly, being the oldest. Adults go through the reproductive phase where laying of eggs takes place.

Egg Stage: The female black soldier fly deposits eggs in clusters for about 500 in numbers in cracks and crevices near or in decaying matter such as dung, carrion, garbage, or other organic waste. Eggs hatch into larvae within approximately four days. Each egg is oval-shaped, measuring about 1 mm in length and pale yellow or creamy white in color.

Larval Stage: Black soldier fly larvae can grow up to 27 mm in length and 6 mm in width, characterized by a dull whitish color and a small, projecting head with chewing mouthparts. They undergo six instars over a period of about 14 days[30]. During this stage, larvae are voracious feeders. As adults, they do not feed and rely on the fat reserves accumulated during the larval phase[80].

Pupal Stage: Before pupation, fifth instar larvae move away from feeding sites to sheltered areas such as ground vegetation where they undergo pupation. The exoskeleton darkens as the larvae transform into pupae, a process that takes approximately two weeks[30].

Adult Stage: Adults are black or blue with a metallic sheen and distinctive translucent "windows" on their first abdominal segment, measuring 15–20 mm in length[102]. They have three-segmented, elongated antennae and white-tipped legs. Two days after emerging, adults mate with males intercepting female's mid-flight, often in lekking areas where males compete for territories[113]. Females lay about 500 eggs in moist organic matter, preferring natural sites like carrion or agricultural environments rich in livestock waste. In urban settings, they adapt by laying eggs in dumpsters or compost heaps that replicate the characteristics of organic habitats.

Factors affecting Growth of Black Soldier Flies

Environment and Space: The population and growth of black soldier fly are affected by space and environment. Mating should ideally occur in medium-sized enclosures, for example, a 2m x 2m x 4m cage inside a greenhouse is adequate[19] whereas smaller cages (27cm × 27cm × 27cm) can be used for rearing[97].

Temperature: Temperature significantly impacts black soldier fly reproduction, particularly mating and oviposition. Ideal temperatures for oviposition range from 27.5 to 37.5 °C (81.5 to 99.5 °F), with variations affecting the fitness and life traits of both sexes.

Light: Light quality and type greatly influence black soldier fly mating and larval pupation. Quartz-iodine lamps[127] and specific wavelengths near 440 nm and 540 nm, mimicking natural sunlight, have proven effective in enhancing mating success[99].

Humidity: Humidity primarily affects the larval stage of black soldier flies, with 70% being optimal for all life stages. Proper humidity levels, often regulated by substrate, prevent desiccation and ensure emergence rates of up to 93%[37].

Antimicrobial nature of Black Soldier Fly (BSF)

Black soldier fly (BSF) larvae produce potent antimicrobial peptides (AMPs) and compounds that enable them to thrive in microbe-rich environments, preventing the proliferation of pathogenic bacteria and fungi. Reports suggest that, lipid and peptide extracts from BSF larvae acted against pathogenic microbes such as *Staphylococcus aureus*, *E. coli* and *Candida albicans*[124]. Efficacy against wound pathogens, including resistant strains, has also been observed with methanol extracts[73]. Moreover, BSF frass shows antifungal effects which may help to manage plant diseases. The antimicrobial capacities of secretions, hemolymph, frass etc. may be effective against multi drug resistant ESKAPE pathogens[12,20,91].

Antimicrobial Peptides (AMPs)

Since the discovery of lysozyme by Alexander Fleming in 1922, antimicrobial peptides (AMPs) have played a crucial role in innate immunity. Antimicrobial peptides (AMPs) are naturally occurring small peptides found in various organisms, playing a crucial role in their innate immune systems. They exhibit broad-spectrum activity against bacteria, fungi, parasites and viruses. Given the rise of antibiotic-resistant microorganisms and growing apprehensions regarding antibiotic use, AMPs have emerged as promising alternatives. Their potential applications span medicine, food production, animal husbandry, agriculture and aquaculture, highlighting their versatile and valuable role in addressing microbial challenges in diverse fields.

The antimicrobial peptide database (APD31) lists over 3,240 AMPs as of August, 2020. These peptides typically consist of 10 to 60 amino acid residues with an average net charge of 3.32, predominantly cationic. Some AMPs also exhibit anionic properties due to the presence of acidic amino acids such as aspartic acid and glutamic acid[48,70,98].

Classification of Antimicrobial Peptides (AMPs)

The classification of antimicrobial peptides (AMPs) pose challenges due to their natural diversity. AMPs are typically categorized based on their sources, activities, structural characteristics and amino acid compositions.

Classification based on Sources: Antimicrobial peptides (AMPs) are widely distributed in different sources including mammals, amphibians, insects, microorganisms, plants and marine organisms. Mammalian AMPs, including cathelicidins and defensins which function in immunity, wound healing and infant health via breast milk[116,126]. In amphibians, including frogs, antimicrobial peptides (AMPs) like magainin are synthesized for protection against pathogens[15]. In insects including black soldier flies and bees, AMPs like cecropin and jellein are generated and possess antimicrobial activity with therapeutic potential[125]. Microbial AMPs, such as nisin and gramicidin, are being explored for bioproduction despite challenges like proteolytic degradation[9]. Plant-derived AMPs like defensins and marine peptides such as myticusin-beta exhibit applications in immune enhancement and antitumor activity, highlighting AMPs' wide-ranging biomedical and industrial potential[39,82].

Classification based on Activities: Antimicrobial peptides (AMPs) are classified based on their specific activities including antibacterial, antiviral, antifungal, antiparasitic and anticancer effects. Antibacterial peptides, like nisin and defensins, target a broad range of pathogens, including MRSA and *E. coli*[57]. Antifungal peptides, such as AurH1, combat fungal infections like *Candida albicans* and *Aspergillus flavus*[67] while antiviral peptides, including Epi-1, FuzeonTM etc. inhibit viruses like foot-and-mouth disease virus and HIV[38]. Antiparasitic peptides, such as Epi-1 and Jellein, target parasites like *Leishmania* and *Trichomonas vaginalis*[10,79]. Anticancer peptides, including Tritrpticin, induce cell death and inhibit tumor growth[3]. These peptides hold promise in medical and agricultural applications, with some also exhibiting anti-inflammatory or anti-diabetic properties, though these are classified separately from AMPs.

Classification of AMPs based on Amino Acid-Rich Species: Antimicrobial peptides (AMPs) can be classified based on amino acid composition, each group exhibiting unique mechanisms and targets. Proline-rich AMPs (PrAMPs) like Tur1A disrupt bacterial protein synthesis by targeting ribosomes after entry via the SbmA transporter, showing activity against Gram-positive and some Gram-negative bacteria, with immunostimulatory effects reported[75]. Tryptophan- and arginine-rich AMPs like indolicidin, Triptrpticin and Octa 2 leverage Trp's interaction with lipid bilayers and Arg's charge to enhance

membrane binding, demonstrating broad-spectrum antibacterial action against *E. coli*, *Pseudomonas aeruginosa*, *S. aureus* etc.[115] Histidine-rich AMPs, like HV2, increase bacterial membrane permeability and show anti-inflammatory properties, with modifications enhancing their therapeutic potential[25]. Glycine-rich AMPs, such as attacins and diptericins, influence peptide structure and activate unique microbicidal pathways, with derivatives like GG3 showing promise against Gram-negative pathogens[117].

Classification based on Antimicrobial Peptide Structures: Antimicrobial peptides are categorized into four structural types: linear α-helical peptides, β-sheet peptides, linear extended peptides and peptides combining α-helices with β-sheets[55]. Additionally, more complex structures like cyclic peptides, lasso peptides and thioether-bridged peptides have been reported[46].

Modes of Action of AMPs

Antimicrobial peptides (AMPs) exert their effects through various mechanisms, primarily categorized into membrane-targeting and non-membrane-targeting mechanisms.

Membrane-Targeting Mechanisms: The mechanisms by which AMPs target cell membranes can be elucidated through several models, include the pore and carpet models. The pore model further subdivides into the toroidal pore and barrel-stave models.

- **Toroidal Pore Model*:*** Known as the wormhole model, AMPs in this model embed vertically into the cell membrane, bending to form a ring-shaped pore approximately 1-2 nm in diameter[74]. Examples include magainin 2, lacticin Q and arenicin. Cationic peptides such as TC19, TC84 and BP2 create fluid domains that compromise of membrane integrity[83].
- **Barrel-Stave Model*:*** AMPs aggregate to form multimeric structures that penetrate the lipid bilayer as channels, leading to the efflux of cytoplasmic contents. Severe disruptions can cause cell membrane collapse and subsequent cell death[65]. For instance, Alamethicin functions via this pore-forming mechanism, while protegrin-1 forms stable octameric β-barrels and tetrameric arcs (half barrels) in both implicit and explicit membranes[63].
- **Carpet-Like Model:** Antimicrobial peptides (AMPs) disrupt cell membranes by aligning parallel to them, with hydrophilic ends facing outward and hydrophobic ends interacting with the lipid bilayer, akin to a detergent-like "carpet" mechanism[84]. Peptides such as LL-37 and β-sheet AMPs destabilize membranes through this approach, a process studied using techniques like ATR-FTIR spectroscopy, as seen with cecropin P1, which flattens and destabilizes bacterial membranes[66,101]. AMPs exploit differences in lipid composition among cell types: bacterial membranes are predominantly anionic with lipids like PE, PG and CL; fungal membranes contain ergosterol and anionic lipids like PC and PI while mammalian membranes are less anionic and contain cholesterol. These distinctions enable AMPs to target pathogens selectively[26,58,106].

Non-Membrane-Targeting Mechanisms: AMPs target differences in bacterial and fungal cell wall components, such as lipopolysaccharides, mannoproteins and teichoic acids. This knowledge aids in designing AMPs with greater specificity and lower cytotoxicity[8,81].

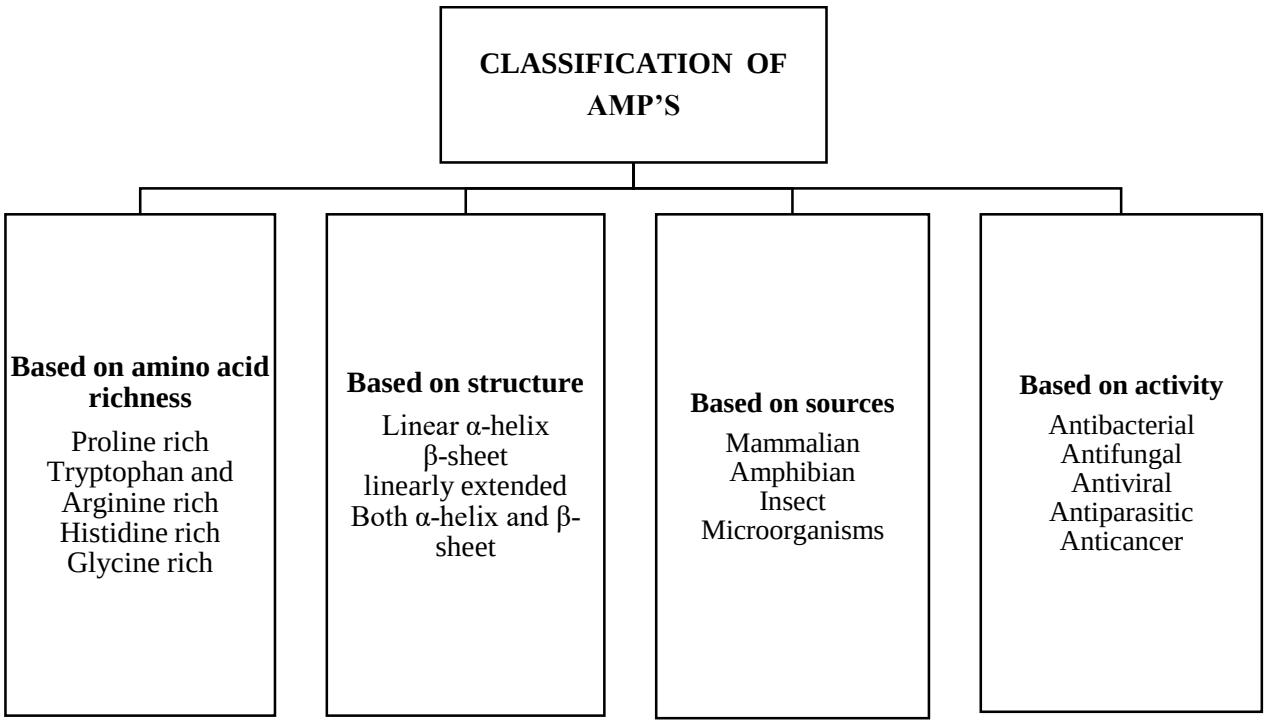

Fig. 1: Classification of AMP's

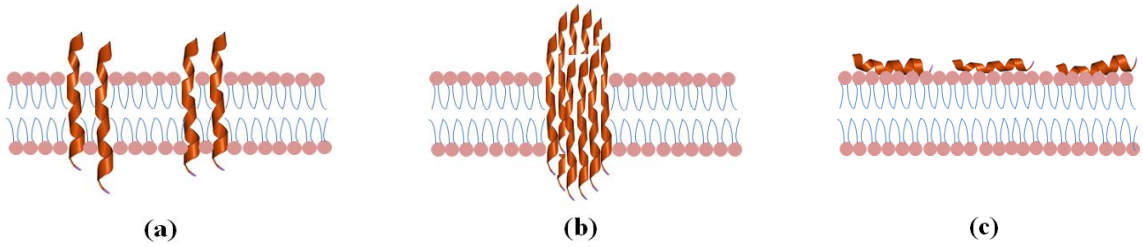

Fig. 2: Mode of action of AMP's (a) Toroidal pore model; (b) Barrel Stave model; (c) Carpet like model

AMPs can also act intracellularly through direct penetration or endocytosis, followed by targeting specific cellular components:

- **Inhibition of Protein Biosynthesis*:*** AMPs interfere with transcription, translation and peptide folding by targeting ribosomes and related enzymes. For example, Bac7 1–35 and Tur1A inhibit protein synthesis in bacteria through distinct mechanisms[71,72]. Some AMPs, like DM3, affect multiple intracellular pathways related to protein biosynthesis. Chaperones play a critical role in AMP selectivity and cytotoxicity prevention by assisting proper protein folding[47,53,120].
- **Inhibition of Nucleic Acid Biosynthesis*:*** AMPs disrupt DNA and RNA synthesis by targeting enzymes like DNA topoisomerase I or binding to specific DNA sites. For instance, indolicidin crosslinks DNA at abasic sites, inhibiting its function[109]. TFP1-1TC24, derived from tongues, degrades nucleic acids after entering target cells[32].
- **Inhibition of Protease Activity*:*** Many AMPs inhibit microbial and host proteases, affecting various metabolic processes. Examples include histatin 5, eNAP-2 and cathelicidin-BF, which inhibit serine proteases and other enzymes[53,104].
- **Inhibition of Cell Division*:*** AMPs disrupt cell division by inducing DNA damage, blocking cell cycle progression, or inhibiting Z-ring formation. For instance, APP and MciZ interfere with *C. albicans* and bacterial cell division respectively[17,59]. AMPs like histatin 5 also affect fungal organelles, inducing reactive oxygen species production and cell death[33].

Environmental Factors affecting the Activity of Antimicrobial Peptides

Metal ions, pH, proteases, salt concentration and temperature significantly influence antimicrobial peptide (AMP) activity. Metal ions like Na^+, Mg^{2+} and Zn^{2+} affect AMP structure, stability and efficacy, with divalent cations generally enhancing antibacterial activity[128]. Environmental pH impacts AMP interactions with bacterial membranes, with some peptides showing heightened activity at acidic or neutral pH levels[36]. Proteases can degrade AMPs, but strategies like encapsulation protect peptides from enzymatic hydrolysis[24]. Salt concentrations affect AMP-membrane binding, with higher salt levels often reducing efficacy due to charge shielding. Lastly, temperature modulates AMP effectiveness by altering membrane fluidity and peptide binding, though extreme heat can denature peptides and impair function[41].

Antimicrobial peptides from *H. illucens* larvae

Insects possess a sophisticated innate immune system comprising of cellular and humoral defense mechanisms. The humoral response involves the synthesis and secretion of antimicrobial peptides (AMPs) from the fat body into the hemolymph[7,35]. AMPs function effectively as antibiotics or fungicides by targeting microbial cell envelopes, particularly the cell membranes and influencing intracellular targets, thereby inducing microbial cell death[65,100].

Recent research has highlighted the antimicrobial potential of *Hermetia illucens* larvae, demonstrating the efficacy of larval hemolymph, maggot extract and secretions against challenging pathogens including multi-resistant "super bugs" like *Staphylococcus aureus* and *Pseudomonas aeruginosa*. These findings are promising for the development of novel antibiotics with therapeutic value. The crude aqueous extracts from *H. illucens* larvae have been investigated for their bioactive effects on biofilms formed by various Gram-positive and Gram-negative microorganisms. Specifically, the research is focused on elucidating the mode of action of *H. illucens* larvae AMPs against *Bacillus subtilis*, a well-established model organism for studying bacterial cell membrane interactions and responses[2].

Classes of AMPs in *H. illucens* Larvae

Hermetia illucens larvae produce a diverse array of antimicrobial peptides (AMPs), encoded by approximately 50 genes, which play a vital role in their innate immunity. These include classes like attacins, defensins, cecropins and diptericins etc. with specific peptides like stomoxynZH1 showing strong activity against pathogens such as *E. coli* and *S. aureus*[114]. The number and type of AMPs vary among insect species, shaped by ecological factors, but their antimicrobial roles are conserved. Studies reveal that bacterial exposure in *H. illucens* larvae triggers increased expression of key AMPs, highlighting their importance in defense mechanisms[96]. Some of the classes are described as follows:

- **Defensins:** Insect defensins are small (~4 kDa), cysteine-rich peptides known for their antimicrobial activity against Gram-positive bacteria, such as *Bacillus subtilis*

and *Staphylococcus aureus* and in some cases, against Gram-negative bacteria and fungi[122]. Structurally, insect defensins consist of six conserved cysteines forming three intramolecular disulfide bonds, adopting a configuration that facilitates interaction with microbial cell membranes, leading to their permeabilization and subsequent cell death[14].

- **Cecropins:** Cecropins are another class of AMPs recognized for their broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria, as well as fungi[122]. Initially isolated from the lepidopteran moth *Hyalophora cecropia*, cecropins function by adopting an amphipathic α-helical structure that interacts with bacterial membranes, disrupting their integrity and leading to cell death[40,49].
- **Attacins:** Attacins are larger (~20 kDa) glycine-rich peptides characterized by their antimicrobial activity primarily against Gram-negative bacteria including *Escherichia coli*[110]. These peptides inhibit bacterial growth by binding to lipopolysaccharides and interfering with outer membrane protein synthesis, thereby increasing membrane permeability and destabilizing the bacterial cell envelope[110].
- **Diptericins:** Diptericins, originally isolated from the dipteran northern blow-fly *Phormia terraenovae*, are unique among AMPs for their glycosylated structure and antimicrobial activity against Gram-negative bacteria like *Escherichia coli* and *Salmonella typhimurium*[23,94]. Despite their glycosylation, diptericins rapidly inhibit bacterial growth without lysing mammalian blood cells, although their precise mode of action remains to be fully elucidated[18].

Extraction of AMPs

Antimicrobial peptides (AMPs) can be extracted from *Hermetia illucens* due to their resilience in contaminated environments and ability to thrive on low-cost feed. The extraction process involves raising larvae through different instars, with fifth-instar larvae being the most productive for AMP synthesis[13,89]. For feed purposes, prepupae are favored due to their higher chitin content. The rearing diet also influences AMP activity, cellulose or protein rich diets enhance activity against Gram-negative bacteria, while diets containing chitin, cellulose, bacteria and plant oil improve efficacy against Gram-positive bacteria[114].

Additionally, larvae starved after exposure to *Lactobacillus* sp. have been studied for their antimicrobial properties[54]. At an industrial scale, cotton seed press cake serves as a cost-effective and sustainable feed option[111]. The steps involved in AMP extraction from BSF larvae are:

- **Immunization of larvae**: Larval immunization involves injecting microorganisms into the hemolymph, typically in their stationary phase to mimic natural infections. This process helps to isolate and analyze antimicrobial peptides (AMPs) and assesses their effectiveness against different microbes. Research has shown that *H. illucens* larvae immunized during the final instar stage produce AMPs with strong activity, particularly against pathogens like *E. coli* and methicillin-resistant *Staphylococcus aureus* (MRSA), while mutilated larvae exhibit the least activity[89]. Studies also highlight that immunization with specific bacteria influences AMP synthesis; for example, *M. luteus* enhances activity against Gram-positive bacteria, while *E. coli* stimulates responses against both Gram-positive and Gram-negative strains. Remarkably, AMPs from *H. illucens* retain their antimicrobial properties even after treatment with enzymes like trypsin or chymotrypsin, especially when larvae are immunized with *L. casei*[54].
- **Maintainence of larvae:** Following the inoculation of microorganisms, larvae are incubated under controlled environmental conditions including temperature, humidity and duration. Even slight adjustments in the homeostasis of an organism can bolster its resilience against stressors[76]. Unlike homothermic animals that can raise their body temperature to combat microbial growth, insects, being poikilothermic, seek warmer environments when infected. Furthermore, heat shock has been shown to accelerate metabolism and the synthesis of defensive molecules[119]. Therefore, storing treated larvae at elevated temperatures is recommended to optimize antimicrobial peptide (AMP) harvesting outcomes.
- **Extraction of AMPs:** Antimicrobial peptides (AMPs) can be extracted from either larval haemolymph or whole larvae. Hetru and Bulet[34] collected haemolymph in pre-cooled tubes containing protease and melanization inhibitors. For smaller insects, liquid nitrogen was used to freeze and grind the specimens into powder, which was then treated with trifluoroacetic acid and inhibitors before centrifugation and filtration. Similarly, haemolymph collection in various studies involved the use of ice-cold tubes containing phenylthiourea crystals to prevent coagulation[89] followed by centrifugation to remove haemocytes.
- A specialized tube was introduced for efficient haemolymph collection. This setup consisted of a 0.5 mL centrifuge tube with a perforated bottom placed inside a 1.5 mL tube. Whole larvae were also homogenized in a methanol, water and acetic acid mixture for AMP characterization. For RNA isolation, larvae were dissected in ice-cold PBS and digestive organs were soaked in lysis buffer before freezing[69]. Another protocol involved grinding larvae in a 20% acetic acid solution, boiling the suspension and then centrifuging it for further analysis[54].

Research on antimicrobial peptides (AMPs) from *Hermetia illucens* larvae reveals various techniques for extraction and the microorganisms these peptides inhibit. Cecropin-like peptide 1, derived from haemolymph, is harvested using solid-phase extraction and reverse-phase chromatography, demonstrating activity against *E. coli*, *Enterobacter aerogens* and *Pseudomonas aeruginosa*[90].

Table 1
Antimicrobial peptides of black soldier fly (*in vitro* verified antimicrobial activity)[50]

S.N.	AMP Family	AMP Example	Activity against
1	Attacin	HI-attacin[103]	*MRSAb* *E. coli* KCCM 11234
2	Cecropin	CLP1[90]	*P. aeruginosa* KCCM 11328 *E. coli* KCCM 11234 *E. aerogenes* KCCM 12177
		Trx-stomoxynZH1a[27]	*S. aureus* *E. coli*
3	Defensin	DLP2[58]	*S. aureus* CICC 546 *S. aureus* ATCC 6538 *S. aureus* ATCC 25923 *S. aureus* ATCC 43300 *S. suis* CVCC 606 *L. ivanovii* ATCC 19119
		DLP3[90]	*S. aureus* KCCM 12256 *S. aureus* KCCM 40881 *MRSAb* *S. epidermis* KCCM 25494 *E. coli* KCCM 11234 *P. aeruginosa* KCCM 11328
		DLP4[56,58,89]	*S. aureus* CICC 546 *S. aureus* ATCC 6538 *S. aureus* ATCC 25923 *S. aureus* ATCC 43300 *S. suis* CVCC 606 *L. ivanovii* ATCC 19119
			S. aureus KCCM 12256 *S. aureus* KCCM 40881 *MRSAb* *S. epidermidis* KCCM 35494 *B. subtilis* KCCM 11316
			S. suis CVCC 3928 *S. epidermis ATCC* 12228 *S. aureus* CVCC 546 *S. pneumoniae* CVCC 2350
		Hidefensin-1[121]	*E. coli*
		Hill_BB_C6571[78] Hill_BB_C7985 Hill_ BB_C16634 Hill_BB_C46948	*E. coli*
		ID13[56]	*S. suis* CVCC 3928 *S. aureus* CVCC 546 *S. pneumoniae* CVCC 2350 *S. epidermis ATCC* 12228
4	Diptericin	Hidiptericin-1[121]	*S. pneumoniae* *E. coli*
5	IATP	HiCG13551[121]	*S. aureus* *S. pneumoniae* *E. coli*

StomoxynZH1, extracted from crushed larvae via Trizol-based RNA extraction, inhibits pathogens like *S. aureus*, *E. coli*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*[27]. Grounded larvae subjected to maceration yield AMPs active against *E. coli*, *P. fluorescens*, *M. luteus* and *B. subtilis*[69]. Directly using larvae as piglet feed enhances the inhibition of *Lactobacilli* and *D-streptococci*[107]. Methicillin-resistant *Staphylococcus aureus* (MRSA) is effectively targeted by AMPs extracted from lyophilized larvae homogenized with acidic methanol[88]. Advanced techniques like treating larvae with liquid nitrogen and isolating RNA using a TRIeasy kit have identified peptides such as Hidefensin-1, Hidiptericin-1 and HiCG13551, which inhibit *Streptococcus pneumoniae*, *E. coli* and *S. aureus*[121]. Finally, maceration of grounded larvae in methanol yields AMPs effective against *Salmonella* and *E. coli*[31].

- **Purification:** Following extraction of AMPs, purification is performed using techniques such as solid-phase extraction, reversed-phase HPLC, size exclusion HPLC, or enzymatic cleavage. Purification aims to eliminate residual salts, synthesis reagents, partially deblocked peptides and truncated peptides[108]. Purified peptides are subsequently subjected to analysis through mass spectrometry or sequencing for further study[88].
- **Storage:** Purified peptides is then stored at freezing temperatures to maintain stability. However, peptides should ideally be utilized immediately post-purification to prevent undesirable alterations.

Applications of Antimicrobial Peptides

Antimicrobial peptides (AMPs) have diverse applications across medicine, agriculture and food industries due to their broad-spectrum antimicrobial properties. In medicine, AMPs are used to treat infections, regulate immune responses and promote wound healing, with peptides like α-defensins showing effectiveness against various pathogens, including viruses and bacteria. AMPs also play a role in dentistry[11], targeting oral diseases and in ophthalmology, offering potential for treating eye infections[43,44]. In wound healing and surgical infections, AMPs like PXL150 show promise for burn and chronic wound care[5]. In the food industry, AMPs serve as natural preservatives, with compounds like nisin and polylysine being FDA-approved for use in food preservation[42]. They are also applied in animal husbandry and aquaculture to improve immunity and combat infections in livestock and fish[4,118].

Additionally, AMPs are used in pearl farming as an alternative to antibiotics[105] and in gene therapy to develop pathogen-resistant plants[77]. While their practical application in agriculture is limited by cost, AMPs offer a potential solution for combating plant diseases and reducing pesticide use[64].

Future Prospects of AMP applications

Advancements in AMP therapeutics focuses on reducing cytotoxicity, enhancing protease stability, combining with antibiotics, inducing precise AMP expression and using engineered probiotics for delivery. Delivery systems such as nanoparticles, hydrogels, creams, ointments and innovative carriers like glutinous rice paper capsules have been developed for optimized wound application[6,112]. Emerging techniques include pheromone-labeled and environment-responsive AMPs (e.g. pH-activated) and nanotechnology-based solutions like nanotubes, graphene and metal nanoparticles to improve targeting and effectiveness[68]. Hybrid peptides, such as PA2–GNU7 targeting the OprF protein in *P. aeruginosa*, highlight cutting-edge innovations in AMP design[43,44].

Conclusion

The rising antimicrobial resistance among microorganisms due to widespread antibiotic use poses a serious global health concern. Efforts to develop new antibiotics are hindered by low profitability, prompting exploration into alternatives across pharmaceutical, agricultural, animal husbandry and food industries. Research on AMPs continues to evolve with extensive data stored in databases. However, understanding the precise mechanisms and optimizing physicochemical properties of AMPs to enhance efficacy, specificity, safety and cost-effectiveness remains a critical area for future exploration[51].

AMPs present promising avenues for combating biofilms and addressing multi-resistant bacterial infections through their diverse mechanisms of action. BSF larvae and their derived products hold significant promise as sources of novel antimicrobial agents, offering potential solutions to combat antibiotic-resistant pathogens and mitigate microbial infections across various sectors. However, distinguishing AMPs from cell-penetrating peptides (CPPs), which have distinct physicochemical properties and mechanisms of action, is crucial for their therapeutic application[21,29,92,93,95]. For instance, SAAP-148, derived from LL-37, demonstrates potent activity against biofilm formation by *S. aureus* and *A. baumannii*[16].

The diversity and efficacy of AMPs found in *H. illucens* larvae underscore their potential for biotechnological applications, particularly in developing novel antimicrobial agents and enhancing agricultural sustainability through reduced pesticide use. While AMPs show immense potential across various medical fields, ongoing research and technological innovations are essential to fully harness their therapeutic benefits and integrate them into clinical practice effectively.

AMPs offer versatile solutions across diverse other sectors, from food preservation to animal husbandry and aquaculture, highlighting their potential to revolutionize practices in these industries while promoting sustainability and safety.

Continued research and innovation are crucial to fully harnessing the benefits of AMPs in these applications. Also,

AMPs hold immense potential for enhancing plant resistance to pathogens and mitigating agricultural losses. Future research should focus on optimizing AMP delivery systems and exploring cost-effective methods to integrate these peptides into agricultural practices effectively. This approach could lead to sustainable solutions for crop protection and food security in the face of increasing global agricultural challenges. Further research into their precise mechanisms and broader applications could pave the way for innovative solutions in medicine, agriculture and beyond.

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