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Revolutionizing agroindustry: Towards the industrial application of antimicrobial peptides against pathogens and pests

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ABSTRACT

Antibiotics are essential chemicals for medicine and agritech. However, all antibiotics are small molecules that pathogens evolve antimicrobial resistance (AMR). Alternatively, antimicrobial peptides (AMPs) offer potential to overcome or evade AMR. AMPs provide broad-spectrum activity, favourable biosafety profiles, and a rapid and efficient mechanism of action with low resistance incidence. These properties have driven innovative applications, positioning AMPs as promising contributors to advancements in various industrial sectors. This review evaluates the multifaceted nature of AMPs and their biotechnological applications in underexplored sectors. In the food industry, the application of AMPs helps to suppress the growth of microorganisms, thereby decreasing foodborne illnesses, minimizing food waste, and prolonging the shelf life of products. In animal husbandry and aquaculture, incorporating AMPs into the diet reduces the load of pathogenic microorganisms and enhances growth performance and survival rates. In agriculture, AMPs provide an alternative to decrease the use of chemical pesticides and antibiotics. We also review current methods for obtaining AMPs, including chemical synthesis, recombinant DNA technology, cell-free protein synthesis, and molecular farming, are also reviewed. Finally, we look to the peptide market to assess its status, progress, and transition from the discovery stage to benefits for society and high-quality products. Overall, our review exemplifies the other side of the coin of AMPs and how these molecules provide similar benefits to conventional antibiotics and pesticides in the agritech sector.

1. Introduction

Antimicrobial resistance (AMR) is an ancient phenomenon (D'Costa et al., 2011) where microorganisms have evolved strategies to adapt to diverse environmental niches (Wright, 2007; D'Costa et al., 2011). The widespread use of antibiotics in healthcare and farming has accelerated AMR through natural adaptation and co-evolution, primarily due to the misuse and overuse of traditional antimicrobials (Prestinaci et al., 2015). Today, the phenomenon of AMR is a latent global problem approaching alarming levels that compromise the value of existing treatments in routine clinical practice and their health outcomes. Impacts of AMR will include increased deaths, unmet demand for livestock products, crop losses, and significant economic and social consequences (Ahmad and Khan, 2019; Oniciuc et al., 2019).

Projections for the health sector indicate that the inability to effectively tackle AMR could lead to more than 39 million deaths directly caused by AMR and 169 million deaths related to AMR between 2025 and 2050 (GBD 2021 Antimicrobial Resistance Collaborators, 2024).

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Healthcare expenditures are expected to increase significantly due to longer hospital stays and extended treatments. Additionally, low- and middle-income countries are likely to experience a substantial economic impact, with a decline in gross domestic product (GDP) exceeding 5 % (GBD 2021 Antimicrobial Resistance Collaborators, 2024; Ho et al., 2024). AMR in livestock and aquaculture is also a growing issue since it can affect animal and human health through the food chain. Notably, over 70 % of antibiotic sales are attributed to their use in animal production for prophylactic purposes and growth promotion (Mehrabi et al., 2020; McNamara and Bomkamp, 2022). The widespread application of pesticides (*e.g.*, bactericides and fungicides) in agriculture has also resulted in negative implications on human health, pollinators, and environmental contamination. In the United States, the economic burden associated with the negative health impacts of pesticide exposure is estimated to exceed US$10 billion annually (Lykogianni et al., 2021).

With fewer novel antimicrobials from the pharmaceutical industry, combating multidrug-resistant microbes is a major scientific, medical, and industrial challenge (Piddock, 2012). The shift in using antimicrobial agents for medicine (*e.g.,* microbial infections, major surgery, organ transplantation, and chemotherapy) to economic sectors (*e.g.,* maintaining health and productivity in livestock production) has increased dependence on antimicrobials (Roope et al., 2019; Caneschi et al., 2023). Projections for 2030 indicate a 67 % rise in antibiotic usage in livestock, a 33 % increase in aquaculture, and an expansion of up to 32 % in human medicine (Baudoin et al., 2021). Despite efforts by different world organizations and public policymakers to develop contingency plans, the discovery of new antimicrobial treatments is more urgent than ever.

Antimicrobial peptides (AMPs) represent a diverse and evolutionarily conserved element of innate immunity, found across multiple life kingdoms (Koehbach and Craik, 2019). In animals, AMPs are produced by skin cells, mucosal surfaces, and immune cells, where they serve as a primary defence mechanism against infectious agents (Sarkar et al., 2021). In plants, AMPs are synthesized as part of their defence responses to bacterial, fungal, and viral pathogens (Shwaiki et al., 2021). Additionally, AMPs produced by fungi and bacteria play a crucial role in protecting these organisms from competing microorganisms, thus aiding in their survival and ecological success (Sugrue et al., 2024). AMPs are seen as an alternative antibiotic modality for the development of leads against pathogens affecting human health and industry (Mejía-Pitta et al., 2021; Torres et al., 2021; Deo et al., 2022; Wan et al., 2024), with ongoing efforts to expand their repertoire. In the Antimicrobial Peptide Database (reviewed on February 13, 2025), 3,306 AMPs isolated from almost all living organisms (bacteria, fungi, mammals, insects, amphibians) have been reported. Furthermore, AMPs display a remarkable diversity of structures and functions. AMPs are frequently less than 50 amino acids typically composed of positively charged and hydrophobic residues (Wan et al., 2024).

Due to their amphiphilic nature, the principal mode of action involves interaction of cationic groups of peptides with negatively charged phospholipids in the cell surface of pathogens, resulting in membrane disruption (Savitskaya et al., 2023). This fast acting mechanism reduces the probability of developing drug resistance (Annunziato and Costantino, 2020). Further, the main target (*e.g.*, membrane) is not directly linked to the genetic code, and therefore in theory, is more difficult for a microbe to evolve resistance mutations (Shukla et al., 2022). AMPs also offer a safer profile (*i.e.*, reduced side effects to host cells) and potent broad-spectrum activity against bacteria, fungi, viruses, parasites, and bioinsecticides. (Windley et al., 2012; Mahlapuu et al., 2016; Annunziato and Costantino, 2020). While AMPs are extensively described in the literature, most studies have focused on their mechanism of action (MOAs) and therapeutic applications in healthcare (Mookherjee et al., 2020). This review aims to highlight advances in AMP applied research, including their multifunctionality, industrial applications, synthesis methods, and market potential, offering insights for their future use in agriculture, aquaculture, farming, and related application fields.

2. Multifaceted nature of antimicrobial peptides

Recent decades have seen a rise in the isolation and characterization of natural, synthetic, and artificial intelligence (AI)-designed AMPs, available in several specific databases (Table 1). First, these growing datasets reveal peptide diversity, ranging from antibacterials to bioactivity against neglected diseases such as *Leishmaniasis*, driven by advances in omics (Morán-Marcillo et al., 2022; Kumar et al., 2024) and computational (Wan et al., 2024) technologies. While most databases focus on wet-lab findings of biological screenings, PepTherDia (D'Aloisio et al., 2021) lists approved peptide-based therapeutics. These libraries provide a snapshot of the potential of AMPs and their biochemical nature, such as primary structure and physicochemical parameters, as well as essential data for algorithm training, which offers potential to transform AMP drug discovery (Robles-Loaiza et al., 2022).

While AMPs focus primarily on drug development, their potential extends across a range of industries. Like a "prism splitting light", the structural plasticity of AMPs affords a diverse array of applications. (Fig. 1). However, advancing their industrial application requires research into their chemical and physical properties, and MOA. We will now critically review the advantages and disadvantages of AMPs, using focused examples, to assess their future potential within industrial biotechnology.

2.1. Antibacterial AMPs

Antibacterial peptides, a subclass of AMPs, are an emerging success stories in terms of approval by drug regulatory agencies. Significantly, antibacterial peptides provide a broad spectrum of action against both Gram-positive and Gram-negative bacteria (Datta et al., 2024), including multidrug-resistant pathogens and progress to early-phase clinical trials. For peptide screening, ESKAPE pathogens are usually the main targets (Magana et al., 2020). This acronym is derived from the scientific names of six highly virulent and antibiotic-resistant bacteria–*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter spp.*– which cause most common nosocomial infections and are becoming increasingly resistant to clinical antibiotics (Navidinia, 2016; Mulani et al., 2019). In addition, ESKAPE pathogen infections are associated with higher morbidity and mortality (Mulani et al., 2019). However, natural, synthetic, and AI-based AMPs inhibit ESKAPE pathogens in both *in vitro* and *in vivo* models (Deslouches et al., 2015; Pletzer et al., 2018; Porto et al., 2018; Bermúdez-Puga et al., 2023b; Maasch et al., 2023). Dermaseptin-SP2, isolated from *Agalychnis spurrelli*, inhibits oxacillin-resistant *Staphylococcus aureus* (ORSA) and *K. pneumoniae* with minimum inhibitory concentrations (MIC) of 2.68 μM and 10.72 μM, respectively (Proaño-Bolaños et al., 2019). Also, dermaseptin-SP2 does not induce red blood cell lysis at MIC concentrations (Proaño-Bolaños et al., 2019). Similarly, lucilin, a 40-amino-acid mature peptide, was active against *Enterobacter cloacae* and clinical isolates of *Escherichia coli.* Importantly, lucillin has neither hemolytic nor cytotoxicity activity (Téllez et al., 2018).

The MOA of antibacterial peptides have been investigated through microscopy analysis (Aslam et al., 2022; Chen et al., 2023a), spectrophotometric-based assays (Maasch et al., 2023), and omics approaches (Schäfer and Wenzel, 2020; Mulkern et al., 2022; Shi et al., 2022). These studies categorize antibacterial peptides into two main classes: membrane-active and intracellular-targeting peptides (Benfield and Henriques, 2020). The most studied are membrane-disrupting peptides, which exert their antibacterial effects by electrostatic interactions, where the positively charged amino acids interact with the negatively charged phospholipids of the bacterial membrane. Later, antibacterial peptides insert into the membrane, causing structural disruptions through three distinct mechanisms such as barrel-stave,

Table 1
List of centralized platforms for AMP discoveries. Many centralized databases have emerged, summarizing the key advances and providing valuable information that supports the development of prediction tools, scientific projects and novel discoveries.

Database	Content in web server	Function	Link (accessed in February 2025)
APD3	3,257 AMP sequences	Antibacterial, antibiofilm, antiviral, antifungal, antiparasitic, anticancer, antidiabetic, insecticidal, wound healing, chemotactic, anti-inflammatory, spermicidal, antioxidant and ion channel activities, protease inhibition	https://wangapd3.com/main.php
$CAMP_{R3}$	8,164 AMP sequences, 757 structures, and 2,083 patents	Antibacterial, antiviral, antifungal and antiparasitic activities	http://www.camp3.bicnirrh.res.in/
DRAMP 4.0	11,612 AMPs, 17,886 patents, 96 AMPs in clinical trials, and 377 stapled AMPs	Antibacterial, antifungal, antiviral, anticancer, antiprotozoal, antiparasitic and insecticidal activities	http://dramp.cpu-bioinfor.org/
LAMP	7,824 Natural and 15,429 synthetic AMPs	Antibacterial, antiviral, antifungal, antiparasitic and anticancer activities	http://biotechlab.fudan.edu.cn/database/lamp/
YADAMP	2,525 AMP sequences	Antibacterial activity	Non accessible
BaAMPs	237 Biofilm-active AMPs	Antibiofilm activity	Non accessible
DBASSP	16,403 Monomeric AMP	Antibacterial, antifungal, antiviral, anticancer, antiprotozoal, antiparasitic and insecticidal activities	https://dbaasp.org/
DADP	2,571 Anuran defence peptide	70 different activities, including antimicrobial activity	http://split4.pmfst.hr/dadp/
AVPdb	2,683 Antiviral peptides	Antiviral activity	http://crdd.osdd.net/servers/avpdb/
HIPdb	981 HIV inhibiting peptides	Anti-HIV activity	http://crdd.osdd.net/servers/hipdb/
Leishmania database	140 Antileishmanial peptides	Anti-leishmanial activity	https://github.com/albert-robles1101/Leishmania-database
BACTIBASE	230 Bacteriocins	Antibacterial activity	http://bactibase.hammamilab.org/main.php
AMPDB v1	59,122 peptides	Antibacterial, antifungal, antiviral, anticancer, antiprotozoal, antiparasitic and insecticidal activities	https://bblserver.org.in/ampdb/ampdb-home
Hemolytik	2,970 Haemolytic and non-haemolytic peptides	Haemolytic activity	http://crdd.osdd.net/raghava/hemolytik/
PepTherDia	105 Approved peptides	Therapeutic and diagnostic applications	http://pepthera.herokuapp.com/

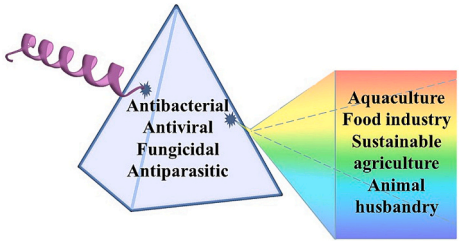

Fig. 1. The biological spectrum activities of AMPs form the basis for their applications in various industrial sectors. Like a prism splitting light, peptides offer a versatile molecular tool, and cost-effective solution for pathogen-related challenges in aquaculture, food, agriculture, and animal husbandry industries.

carpet, or toroidal-pore mechanisms (Espeche et al., 2024). The heterogeneous composition of the membrane plays a crucial role in the efficacy, potency and selectivity of antibacterial peptides (Fux et al., 2023). Additionally, several peptides inhibit bacterial growth by interaction with intracellular targets (*e.g.*, DNA, RNA) and therefore disrupt essential cellular processes (*e.g.*, synthesis, replication, and translational process) (Vanzolini et al., 2022). To date, Lytixar (LTX-109) (a peptidomimetic), POL7080 (protegrin-1 analogue), and hLF1–11 (lactoferricin derivative) are key examples of AMPs under clinical trials against bacterial infections (Mahlapuu et al., 2016; Kumar et al., 2020; Ting et al., 2020).

Combinatorial therapy with AMPs and antibiotics offers an alternative strategy against multidrug-resistant bacteria, enhancing potency, reducing dosage and side effects, lowering resistance risk, and broadening activity (Mhlongo et al., 2023). Advantages over monotherapy include higher potency, reduced dose of each drug, fewer side effects, lower risk of drug resistance, and a broader spectrum of action (León-Buitimea et al., 2020). This approach can re-sensitize multidrug-resistant bacteria to antibiotics. For example, treatment of vancomycin-resistant *S. aureus* (VRSA) strains with the synthetic peptides WR-12 and D-IK8 at sub-inhibitory concentrations resulted in suppression of resistance to vancomycin, teicoplanin, and oxacillin (Mohamed et al., 2016).

2.2. *Anti-fungal AMPs*

Fungal infections are a serious threat to human health causing 1.7 million deaths annually. Critically, there are only four antifungal drug classes and the emergence of antifungal drug-resistant strains is a major challenge (Bongomin et al., 2017; Kainz et al., 2020). The assessment of antifungal properties of AMPs offers a potential path to fight fungal infections. Magainins, dermaseptins, defensins (including α- and β-defensins), temporins, cecropins, and halocidin-derivatives all show activity against pathogenic fungi, such as *Aspergillus spp., Candida albicans*, and non-*albicans Candida* species (Jang et al., 2006; Bondaryk et al., 2017; Fernández de Ullivarri et al., 2020; Huan et al., 2020). Recently, cruzioseptin-1, isolated from *Cruziohyla calcarifer*, inhibited *in vitro* the growth of *C. albicans* with only 1 % hemolysis at its MIC (3.77 μM) (Proaño-Bolaños et al., 2016).

The main MOA for antifungal peptides is a pore-forming model similar to that observed in bacteria, inhibition of cell wall synthesis, and targeting intracellular components (Buda De Cesare et al., 2020; Struyfs et al., 2021). A synthetic AMP inspired from plant proteins induced a 3.6 kDa pore in the membrane of *Cryptococcus neoformans* (Aguiar et al., 2022). The plant defensin NaD1 kills fungal species by interacting with chitin and β-glucan on the cell surface, which compromises cell wall integrity (Bleackley et al., 2019). In addition, transcriptomic analysis of *C. albicans* exposed to NpRS indicates inhibition of protein synthesis (Li et al., 2023). However, the similarity between fungal and mammalian cell membranes is a challenge for identifying promising lead peptides, due to low specificity (Vanzolini et al., 2022). Several AMPs exhibit *in*

vivo activity against *C. albicans*. Jelleine-I (from *Apis mellifera*) treatment (10 mg/kg) enhanced the survival rate and showed low acute toxicity (LD_{50} > 1000 mg/kg) in mice infected with *C. albicans* (Jia et al., 2018). Also, K10S reduced the mortality of *Galleria mellonella* infected with *C. albicans* (Ciociola et al., 2021). hLF1–11, Novexatin (NP213), CZEN-002, and PAC-113 are further examples of antifungal peptides tested in clinical trials (Mahlapuu et al., 2016; Ting et al., 2020).

2.3. Antiviral AMPs

Viruses are a major cause of human diseases with limited small molecule therapeutic options. The influenza virus, human immunodeficiency virus (HIV), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are widely recognized for their association with high morbidity and mortality rates (Elnagdy and AlKhazindar, 2020; Wu and McGoogan, 2020). Several peptides with anti-HIV activity have been reported (Vilas Boas et al., 2019). These include caerin 1.1, caerin 1.9, magainin 2, ranatuerin-2P, dermaseptin S1, and maculatin 1.1 from amphibian skins (VanCompernolle et al., 2005; Madanchi et al., 2020), cecropin A and melittin from insects (Wachinger et al., 1998), defensins, protegrin analogs, and cathelicidin from mammals, birds, fish and reptiles (Tamamura et al., 1995; Wang et al., 2008a; Madanchi et al., 2020). Fuzeon (enfuvirtide), a 36-amino acid peptide, is the first anti-HIV drug approved by the U.S. Food and Drug Administration (FDA) (Fung and Guo, 2004).

Coronaviruses have caused two major epidemics: severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV). COVID-19, which is caused by SARS-CoV-2, was declared a pandemic by the World Health Organization (Elnagdy and AlKhazindar, 2020; Wu and McGoogan, 2020). SARS-CoV-2 encoded RdRp, 3CL, spike, and nucleocapsid proteins are essential for viral proliferation and infection (Huang et al., 2020; Balmeh et al., 2021). *In silico* methods suggest peptides can inhibit spike protein binding to the angiotensin-converting enzyme 2 cell surface receptor (Baig et al., 2020; Han and Král, 2020; Kurpe et al., 2020; Souza et al., 2020; Sekar and Rajasekaran, 2021). With the same MOA, HD5 (α-defensin) displayed antiviral activity against SARS-CoV-2 *in vitro* (Wang et al., 2020), while 2019-nCoV-HR2P, IBP02, and EK1C4 showed highly potent inhibitory effects against SARS-CoV-2 S-mediated cell-cell fusion (Xia et al., 2020a; Xia et al., 2020b; Zhu et al., 2020).

Several antiviral peptide databases, namely APD (Wang et al., 2016a), DRAMP 2.0 (Kang et al., 2019), AVPdb (Qureshi et al., 2014) and dbAMP (Jhong et al., 2019), list peptides with activity against various viruses, including those causing large economic losses. Predictive tools and AI-based methods (Thakur et al., 2012; Meher et al., 2017; Beltrán Lissabet et al., 2019; Schaduangrat et al., 2019; Chowdhury et al., 2020; Li et al., 2020b), have accelerated antiviral peptide discovery by reducing costs and time. For example, Feijoo-Coronel et al. (2024) integrated *in silico* tools to identify antiviral AMPs within chenopodin, a vegetable protein, revealing AMPs that reduce SARS-CoV-2–induced infection of human pulmonary cells (Feijoo-Coronel et al., 2024). The synergy of *in silico* and *in vitro* approaches refines peptide screening, accelerating the discovery of safe candidates (Feijoo-Coronel et al., 2024). *In vivo* studies of antiviral AMPs will be discussed in Section 3.

2.4. Antiparasitic AMPs

Neglected tropical parasitic diseases primarily affect lower-middle income countries (LMIC), causing more than 1 million deaths per year (Renslo and McKerrow, 2006). The low efficacy and selectivity of the existing treatments for these diseases result in prolonged exposure, leading to significant health and economic impacts, including high costs, serious adverse effects, and increased drug toxicity (Sacks, 2014). AMPs are effective against parasites that cause Chagas disease, leishmaniasis, and malaria (Rivas et al., 2009; Vale et al., 2014; Lacerda et al., 2016; Proaño-Bolaños et al., 2024). Cruzioseptin-1, from amphibian skin secretions, showed dual action against promastigote and amastigote forms of *Leishmania (Viannia) braziliensis* (Mendes et al., 2020). Anti-leishmanial peptides include bombins, magainins, temporins, eumenitin, cathelicidins, decoralyn, and tachyplesin, are compiled in an AMP-database to combat *Leishmania* (Robles-Loaiza et al., 2021). This open repository provides the sequence, source, biological potency (IC_{50}) and biochemical parameters of this class of peptides. Another antiparasitic peptide database is ParaPep, which describes inhibitory activity against *Trypanosoma*, *Leishmania*, and *Plasmodium* genera (Mehta et al., 2014).

The MOAs of antiparasitic AMPs is (1) plasma membrane disruption or (2) intracellular targets (El-Dirany et al., 2021; Rojas-Pirela et al., 2023; Hagemann et al., 2024). Members of the dermaseptin and temporin families targets *Trypanosoma cruzi* via membrane disruption (Pinto et al., 2013; Raja et al., 2017). Similarly, a 4-amino acid peptide (KDEL) inhibits *Leishmania tarentolae* through membrane disruption, leading to apoptosis (Cao et al., 2019). Mastoparan, a peptide isolated from the wasp venom *Polybia paulista*, kills *T. cruzi* by targeting glyceraldehyde-3-phosphate dehydrogenase from glycolysis (Vinhote et al., 2017). While potentially useful in the future, research into antiparasitic AMPs remains limited. A disadvantage is peptide activity against intracellular forms of parasites, which complicates their potential application in the clinic (Proaño-Bolaños et al., 2024).

3. The other side of the coin: Alternative applications of AMPs for industrial biotechnology

Advancements in peptide therapeutic and clinical approval provide the basis for broader application for industrial biotechnology (Gare et al., 2025). Key challenges for biochemists, medicinal chemists, chemists, and bioinformaticians, include untangling the complex structure-activity relationships of AMPs, as well addressing scalable and cost-effective production of these molecules. While AMPs studies have focused on pharmaceutical drugs, nonetheless, new niches for AMPs are emerging, including aquaculture, food industry, agriculture, and animal husbandry. To accelerate the development of AMPs, this review aims to evaluate their current progress, with a specific focus on the application of AMPs for the agritech sector.

3.1. Potential uses of AMPs for treating diseases in aquaculture

World aquaculture production reached 82 million tonnes in 2018, valued at US $250 billion. Due to intensification of the aquaculture industry, infectious diseases have emerged causing serious economic impacts (Elgendy et al., 2024). There is also an overreliance on antibiotics, chemotherapeutics, and vaccines for disease prevention. These strategies have limited efficacy, kill beneficial microbiota, and select for multidrug-resistance (Falco et al., 2012; Pérez-Sánchez et al., 2018). Therefore, a key priority involves the development of novel control approaches within aquaculture. Several reviews describe key pathogens affecting marine invertebrates (Destoumieux-Garzón et al., 2016; Pérez-Sánchez et al., 2018). Prevalent bacterial species in aquaculture diseases include *Vibrio, Aeromonas*, and *Pseudomonas* genera. We will next discuss examples of AMPs for reducing the burden of aquaculture diseases (Fig. 2). Key strategies include genetic engineering, peptide inoculation, and the incorporation of peptides into fish pellets.

There are several reported AMPs with selective activity against fish pathogens *in vitro*. The piscidins, pleurocidins, and hepcidins inhibit the growth of *Vibrio spp.* (Cole et al., 1997; Jia et al., 2000; Huang et al., 2007; Ruangsri et al., 2012; Umasuthan et al., 2016). Additionally, piscidins and pleurocidins inhibit *Aeromonas salmonicida* (Jia et al., 2000; Douglas et al., 2003; Patrzykat et al., 2003; Samuelsen et al., 2006; Noga et al., 2009). Gs1, Gs2, Pp1, and Pp3, belong to the grammistin family, are antibacterial against *A. salmonicida, Photobacterium damselae* subsp. *piscidida, Vibrio anguillarum,* and *Vibrio parahaemolyticus* with MIC values between 6.25 and 50 μg/mL (Yokota et al., 2001).

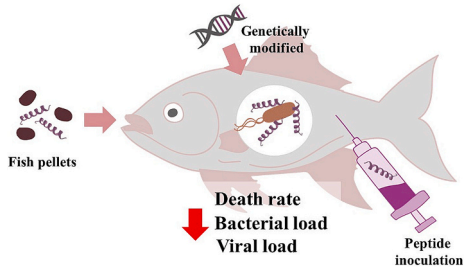

Fig. 2. AMP control aquaculture pathogens. The recombinant expression, injection, or incorporation of AMPs into fish pellets has demonstrated beneficial effects in pathogen load reduction. These strategies leverage the selective killing properties of AMPs, which selectively target bacteria and viruses, while ensuring the health of fish remains unaffected.

Mixinidin, a dodecapeptide isolated from *Myxine glutinosa,* is bioactive against *A. salmonicida* subsp. *salmonicida*, *Listonella anguillarum*, and *Yersinia ruckeri* in the concentration range of 1–2 μg/mL. Importantly, mixinidin does not induce hemolysis in rabbit red blood cells (Subramanian et al., 2009). Additionally, some AMPs retain *in vivo* antibacterial activity. Treatment of common carps (*Cyprinus carpio*) infected with *V. anguillarum* with Tf-hep (4 μg/fish), a hepcidin found in *Trachidermus fasciatus*, increased survival rates (Liu et al., 2017b). Hepcidin-25 was also tested *in vivo* in grass carp. The injection of these peptides before or after the *A. hydrophila* infection significantly improved the survival rate of this species (Hu et al., 2020).

Supplementation of AMPs in the basal diet has been reported to produce beneficial effects on fish (Dong et al., 2015; Su et al., 2019; Liu et al., 2020b; Li et al., 2020c; Rashidian et al., 2021; Liu et al., 2022). The addition of an AMP (200 mg/kg) to *Micropterus salmoides* diet increased growth performance, antioxidant capacity, and immunity compared to the control group (Li et al., 2020c). The use of cecropin as a feed additive for common carp (*Cyprinus carpio*) had similar results (Dong et al., 2015).

Several transgenic fish have been genetically engineered to express AMPs to resist bacterial diseases (Table 2). Transgenic medaka, which contained a cecropin construct, showed a reduction in cumulative mortality caused by *P. fluorescens* and *V. anguillarum* (Sarmasik et al., 2002). Addition of the cecropin gene into the channel catfish genome gave resistance to *Edwardsiella ictaluri* and *Flavobacterium columnare* (Dunham et al., 2002). Similarly, transgenic rainbow trout expressing cecropin P1 or *CF*-17 exhibited resistance to *A. salmonicida* (Chiou et al., 2014). However, a potential issue with transgenic cell lines is pleiotropy and unexpected side-effects. Transgenic zebrafish expressing TP3 were resistant to *Streptococcus agalactiae* infection but also displayed a color change (orange-red) in the body (Su et al., 2018).

Viral diseases are a major cause of mortality in the aquaculture industry (Kibenge, 2019). Singapore Grouper Iridovirus (SGIV) is the causative agent of extremely high mortality rates (>90 %) in cultured grouper fry (Li et al., 2020a). Nervous Necrosis Virus (NNV) is lethal pathogen to several economically relevant fish species (Toffan et al., 2017). Viral Hemorrhagic Septicaemia Virus (VHSV) causes an annual loss of about 50 million euros in Europe (Micol et al., 2005). Some studies show AMPs uses against aquaculture viruses. Ec-defensin from *Epinephelus coioides* was active against SGIV and NNV by inhibiting the infection and replication (Guo et al., 2012). HNP1 was active against VHSV through inactivating viral particles and inducing cellular antiviral responses in host cells (Falco et al., 2007). Hepcidins are small cysteine rich molecules that inhibit Infectious Pancreatic Necrosis Virus (IPNV), White Spot Syndrome Virus (WSSV), SGIV, *Siniperca Chuatsi* Rhabdovirus (SCRV), and largemouth bass *Micropterus Salmoides* Reovirus (MsReV) (Wang et al., 2009; Rajanbabu and Chen, 2011; Zhou et al., 2011; Cai et al., 2012; Gui et al., 2016). Additionally, several studies report the activity of AMPs *in vivo* (Table 2). SmHep1P and SmHep2P, isolated from *Scophtalmus maximus*, reduce the viral load of

Table 2
Current advances in *in vivo* applications of AMPs for the effective disease management in aquaculture. AMPs have been employed across various fish species, significantly reducing pathogen-induced mortality and ensuring higher survival rates.

AMP	Target fish	Pathogen	Beneficial effects	Ref.
Hep1	*Dicentrarchus labrax*	*Vibrio vulnificus*	Reduction in mortality	(Álvarez et al., 2016)
Tf-Hep	*Cyprinus carpio*	*Vibrio anguillarum*	Reduction in mortality	(Liu et al., 2017b)
Hepcidin-25	*Ctenopharyngodon idella*	*Aeromonas hydrophila*	Treatment with peptides before or after bacterial infection reduced mortality rate	(Hu et al., 2020)
SmHep1P and SmHep2P	*Scophthalmus maximus*	*Edwardsiella tarda*	Reduced bacterial load in kidney, liver, and spleen	(Zhang et al., 2014)
SmHep1P and SmHep2P	*S. maximus*	Megalocytivirus	Reduced viral load in kidney, liver, and spleen	(Zhang et al., 2014)
AN-hepc	Zebrafish (*Danio rerio*)	*V. vulnificus* or *Streptococcus agalactiae*	Reduction in mortality	(Chi et al., 2015)
CEME	Coho salmon (*Oncorhynchus kisutch*)	*V. anguillarum*	Reduction in mortality	(Jia et al., 2000)
Pleurocidin amide	Coho salmon (*O. kisutch*)	*V. anguillarum*	Reduction in mortality	(Jia et al., 2000)
TH1–5 and epinecidin-1	Grouper (*Epinephelus coioides*)	Nervous necrosis virus	Co-treatment with TH1–5 or epinecidin-1 enhanced grouper survival rate	(Wang et al., 2010b)
TH1–5 and epinecidin-1	Medaka (*Oryzias latipes*)	Nervous necrosis virus	Co-treatment, pretreatment, or post-treatment with both peptides reduced fish mortality	(Wang et al., 2010b)
g-ple 22–42	Tilapia (*Oreochromis mossambicus*) or grouper (*E. coioides*)	*V. vulnificus*	Co-treatment or pretreatment with g-ple 22–42 enhanced survival rate	(Pan et al., 2007)
NKL-24	*Patinopecten yessoensis*	*Vibrio parahaemolyticus*	Pretreatment or post-treatment with NKL-24 enhanced survival rate	(Shan et al., 2020)
Cecropin	Transgenic medaka (*O. latipes*)	*Pseudomonas fluorescens* or *V. anguillarum*	Transgenic fish expressing cecropin transgene showed reduction in cumulative mortality	(Sarmasik et al., 2002)
Cecropin B	Transgenic channel catfish (*Ictalurus punctatus*)	*Edwardsiella ictaluri*	Transgenic fish expressing cecropin B transgene showed reduction in cumulative mortality	(Dunham et al., 2002)
Cecropin P1 or *CF*-17	Transgenic rainbow trout (*Oncorhynchus mykiss*)	*Aeromonas salmonicida* or Infectious hematopoietic necrosis virus	Transgenic fish expressing TP3 transgene showed reduction in bacterial or viral load in the examined tissue	(Chiou et al., 2014)
TP3	Transgenic zebrafish (*D. rerio*)	*S. agalactiae*	Transgenic fish expressing cecropin P1 or *CF*-17 transgene showed reduction in cumulative mortality	(Su et al., 2018)

megalocytivirus in turbot (Zhang et al., 2014). A combinatorial injection of TH1–5 and epinecidin-1 into medaka or grouper provides protection against NNV infection (Wang et al., 2010a; Wang et al., 2010b).

Parasites present a significant threat to the fish industry, with no effective treatments currently available. AMPs are active against several aquaculture parasites. Piscidin 2, Pis1, and Pis2 were lethal against *Trichodina sp., Cryptocaryon theront, Amyloodinium ocellatum*, and *Ichthyophthirius theront* (Colorni et al., 2008; Ruangsri et al., 2012). Likewise, HLP-1 has activity against *Amyloodinium ocellatum* (Noga et al., 2001). Similarly, Of-Pis1 showed antiparasitic activity against *Miamiensis avidus* with a IC_{50} of 0.4 μM (Umasuthan et al., 2016). Finally, EcPis-3S isolated from *E. coioides*, was active against *Cryptocaryon irritans* and disrupts the cell membrane (Zhuang et al., 2017). Table 2 presents a compilation of peptide candidates assessed in *in vivo* studies, highlighting their protective effects against pathogens. We have included details on the fish species used, the pathogens involved, and the beneficial effects observed.

This section highlights the multifunctional benefits of AMPs, as well as current limitations, for protecting aquatic animals from microbial pathogens. Their use in aquaculture could diversify disease management strategies, increasing productivity and reducing outbreaks, thus supporting economic stability and food security. While further research is needed, the potential prospects of AMPs in aquatic farming is promising and could reduce our overreliance on small molecule chemical-based treatments, which are associated with AMR and toxicity.

3.2. AMPs in the food industry

Food spoilage is a significant economic and environmental problem for the food industry (Shwaiki et al., 2020c). About 1.3 billion tons of food is lost to food spoilage every year. Food spoilage is caused by microbes including bacteria and fungi, present at every stage of the food supply chain and cause bad odors and flavors (Odeyemi et al., 2020). Several physical factors influence microbial-based food deterioration, including temperature, pH, and water content (Pinu, 2016; Odeyemi et al., 2020).

Food-poisoning microorganisms are another problem in the food industry. Many species contaminate food and produce toxic chemicals harmful to human health (Vishweshwaraiah et al., 2021), with some life-threatening (Chlebicz and Śliżewska, 2018). In 2010 the World Health Organization reported about 600 million individuals contracted foodborne illnesses, with half attributed to pathogenic bacteria, including *Campylobacter spp.*, *Salmonella spp.*, and *Listeria spp.* 2 million cases of campylobacteriosis and salmonellosis are reported each year in the United States. Listeriosis, caused by *Listeria monocytogenes*, has a 30 % mortality rate (Cusimano et al., 2019; Bahrami et al., 2020). Additionally, food poisoning impacts the economy. The United States spends about $83 billion USD to minimize food poisoning, while Australia and New Zealand spend $1 billion USD and $86 million USD, respectively (Chlebicz and Śliżewska, 2018).

Food preservation techniques include heat treatment, pH, drying, and chemical preservatives. However, this approach can produce undesirable effects (Chacha et al., 2021). Heat treatment can produce unwanted organoleptic and nutritional properties, while long-term synthetic chemical preservatives can adversely affect consumer health (Tiwari et al., 2009; Sharif et al., 2017). Therefore, there is an interest in using natural compounds, including AMPs (Bermúdez-Puga et al., 2023a; Bermúdez-Puga et al., 2024; Chen et al., 2023b; Nisa et al., 2023), to increase the shelf-life and safety of food products.

Several AMPs inhibit food spoilage microorganisms and food poisoning pathogens. D-lp1, a 47 amino acid peptide isolated from barley endosperm, inhibits spoilage microorganisms such as *Zygosaccharomyces bailii, Zygosaccharomyces rouxii, Saccharomyces cerevisiae,* and *Debaryomyces hansenii* (Shwaiki et al., 2020c), and snakin-1 shows anti-yeast activity against *Z. bailli* Sa1403Z and *D. hansenii* CBS2334 (Shwaiki et al., 2020b). Enrique et al. (2007) tested several antifungal peptides. PAF36, PAF26 and its D-enantiomers inhibited the growth of *S. cerevisiae, Z. bailii,* and *Zygosaccharomyces bisporus*, while López-Solanilla et al. (2003) reported that human defensin 1, human defensin 2, thionin, snakin, and magainin inhibited *L. monocytogenes*.

In addition to antimicrobial activity, it is essential to assess the toxicity of peptides through assays, such as hemolysis or cell viability. At MIC, snakin (100–200 μg/mL) and Rs-AFP1 (25–50 μg/mL) induced less than 10 % hemolysis (Shwaiki et al., 2020a; Shwaiki et al., 2020b), while Rs-AFP1 increased Caco-2 cell viability (Shwaiki et al., 2020a; Shwaiki et al., 2020b). Peptidases degradation in the human gastrointestinal tract is another safety consideration; for instance, α-chymotrypsin degrades Rs-AFP1 and Rs-AFP2 (Shwaiki et al., 2020a).

For preservative use, AMPs must be stable under various physical and chemical conditions. D-lp1 retained activity against *Z. bailii* at 100 °C or pH 3–7 (Shwaiki et al., 2020c), while Rs-AFP1 and Rs-AFP2 were only active at pH 5 or 100 °C for a maximum of 15 min (Shwaiki et al., 2020a). In a related example, KK14 was stable at pH 3.5–6.5, but its MIC increased four-fold at pH 9.5 (Thery et al., 2019).

Several AMPs have been tested in various food systems (Table 3) including beverages, meat, and dairy products (Fig. 3). Snakin-1 inhibited *Z. bailii* in Fanta Orange and cranberry juice (Shwaiki et al., 2020b), while D-lp1 was effective against *Z. bailii* in apple juice and Fanta orange, but less so in wine (Shwaiki et al., 2020c). Dermaseptin K4-S4 reduced *E. coli* O157:H7 viability in apple juice within 2 h (Yaron et al., 2003).

Several AMPs are currently available on the food market. Nisin is a ribosomally-synthesized and post-translationally modified peptide and a member of the bacteriocin family of AMPs (Field et al., 2023). Nisin is derived from *Lactococcus lactis* subsp. *lactis* and is used widely as a food preservative. The U. S. FDA classifies nisin as Generally Regarded As Safe (GRAS) and it is used in >80 countries (Santos et al., 2018; Soltani et al., 2021). The Food and Agriculture Organization of WHO (FAO/WHO) advises the food industry should contain a maximum of 2.5 % (*w*/w) of active nisin with an activity of 10^6 arbitrary units (AU)/g (Müller-Auffermann et al., 2015). Similarly, pediocin PA-1, a bacteriocin isolated from *Pediococcus spp.*, is another commercially available biopreservative acting against foodborne pathogens (Negash and Tsehai, 2020).

In summary, while there are still only limited examples of AMP-based strategies for the food industry, the success of nisin provides evidence for their application and biological safety. Significantly, linear AMPs will withstand high temperatures during food production and then will readily degrade via human proteolytic enzymes in the gut, whereas small molecule chemicals are processed in the liver for excretion. We anticipate that the application of AMPs for food security is set to grow and potentially provides a safer alternative to chemical and physical-based food preservation approaches.

3.3. The potential of AMPs for sustainable agriculture

Bacteria, fungi, viruses, and insects are responsible for up to 40 % of crop production losses (Flood, 2010). Currently, agrochemicals and fungicides are used, although these are linked to significant environmental and human health issues, and rising resistance (Guo et al., 2021). This has led to a decline in their use (Hamrouni et al., 2024), leading to a growing demand for next-generation biopesticides that align with One Health principles (McEwen and Collignon, 2018; Fisher et al., 2022; Hoffmann et al., 2022). Therefore, there is a growing interest in the use of AMPs for crop protection. Several studies are focusing on developing disease-resistant plants expressing AMPs (Gao et al., 2000; Sharma et al., 2000; Muñoz et al., 2007; Chahardoli et al., 2018) derived from microbes, plants and animals, or by peptide engineering (Table 4).

A synthetic peptide fusion of dermaseptin B1 and chitin-binding domain (CBD) from Avr4 were expressed and isolated from transgenic plants, and shown to inhibit the growth of *Alternaria alternata*, *Fusarium oxysporum*, and *Fusarium solani* (Khademi et al., 2020). LL-37 peptide, an

Table 3
Overview of AMPs and their target pathogens in the food industry. Most studies have concentrated on beverages such as soda, wine, and juice, while fewer have explored the application of AMPs in yogurt, patties, port, shrimp and vegetables. A broad range of pathogens has been evaluated, showcasing the effectiveness of AMPs.

AMP	Food/drink	Pathogen	Beneficial effects	Ref.
D-lp1	Apple juice, Fanta Orange and wine	*Zygosaccharomyces bailii*	Total pathogen inhibition in apple juice and Fanta Orange; partial inhibition in wine	(Shwaiki et al., 2020c)
Snakin-1	Fanta Orange, cranberry juice and apple juice	*Z. bailii*	Pathogen inhibition in 3 different drinks	(Shwaiki et al., 2020b)
Rs-AFP1 or Rs-AFP2	Fanta Orange, cranberry juice, orange juice, apple juice and salad dressing	*Z. bailii*	Both peptides were effective in inhibiting the pathogen in Fanta Orange, apple juice and cranberry juice. However, peptides were ineffective in orange juice	(Shwaiki et al., 2020a)
PAF26L or $LfcinB_{17–31}$	White wine	*Saccharomyces cerevisiae, Z. bailii* or *Zygosaccharomyces bisporus*	LfcinB17–31 partially inhibited the growth of *Z. bisporus* in wine in an inoculum-dependent manner	(Enrique et al., 2007)
KK-14, dKK-14, Dip KK-14, or KK-14 (R10)	Fanta Orange and mayonnaise	*Z. bailii*	All peptides inhibited *Z. bailii* in Fanta Orange.	(Shwaiki et al., 2019)
KK-14, kk-14, KK-14 (Dip), or KK-14 (R)	Apple juice	*Aspergillus niger*	KK-14 (Dip) and KK-14 (R) reduced fungal growth in apple juice	(Thery et al., 2019)
Plantaricin K25	Kimchi	*Bacillus cereus*	Plantaricin K25 gradually reduced viable colony-forming units of *B. cereus* in kimchi	(Wen et al., 2016)
Dermaseptin K4-S4	Apple juice	*Escherichia coli* O157:H7	Peptide reduced bacterial growth in apple juice	(Yaron et al., 2003)
Bacteriocin	Shrimp	*Listeria monocytogenes*	Treatment with bacteriocin reduced the growth of L. *monocytogenes*	(Ladha and Jeevaratnam, 2020)
MccJ25(G12Y)	Yogurt	*Salmonella enterica* Enteritidis (CECT 4396), *E. coli* O157:H7 (NCTC 12900), or *Enterobacter cloacae* (CECT 194)	MccJ25(G12Y) significantly inhibited the growth of the 3 pathogens	(Corbalán et al., 2021)
MccJ25(G12Y)	Ground beef patties	*E. coli* O157:H7	MccJ25(G12Y) reduced bacterial growth in ground beef	(Corbalán et al., 2021)
MccJ25(G12Y)	Fish patties	*E. cloacae*	Treatment showed a reduction in bacterial load	(Corbalán et al., 2021)
Mytichitin-CB	Pork	*Staphylococcus aureus* ATCC 25923, *E. coli* O157 ATCC 35150, or *P. fluorescens* CGMCC 11802	The 3 pathogens were inhibited by the peptide	(Meng et al., 2021)
Pentocin 31–1	Pork	*L. monocytogenes* NICPBP 54002 or *P. fluorescens* CGMCC 1.55	Bacteriocin slightly reduced pathogen survival	(Zhang et al., 2010)
zp37	Alfalfa sprouts	*E. coli* O157:H7	Treatment with zp37 inhibited the pathogen for 7 days	(Yi et al., 2021)

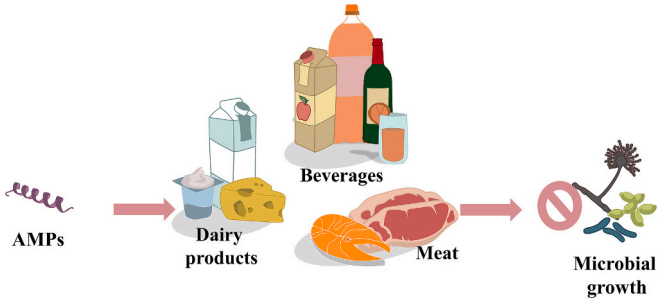

Fig. 3. AMPs as effective preservatives in the food industry. AMPs have primarily been introduced as proof of concept and assessed in various foods, particularly in meats and beverages like juice, soda, and wine. AMPs offer advantages for controlling microbial growth, ensuring safety, and maintaining product quality.

element of the human innate immunity, was inserted into the genomes of tomato (*Solanum lycopersicum*) (Jung, 2013), Chinese cabbage (*Brassica rapa* var. *chinensis*) (Jung et al., 2012), and rice (*Oryza sativa* L. var. *Japonica* cv. *Dongjinbyeo*) (Lee et al., 2017), leading to enhanced plant resistance against microbial plant pathogens. Additionally, tobacco plant expression of a recombinant AMP conferred resistance to fungal pathogens (Khademi et al., 2020). MSrA1, a peptide chimera constructed by NH_2-terminus-modified cecropin and melittin, was integrated into the genome of potato (*Solanum tuberosum* L.). The transgenic potato plants were challenged against two fungal pathogens (*Phytophthora cactorum* and *F. solani*) and a bacterial pathogen (*Erwinia carotovora*). MSrA1 expression in transgenic potatoes conferred resistance to these diseases (Osusky et al., 2000). Collectively, various plant species, AMP sequences, and microbial models are being explored to assess AMP-based GMO expression for sustainable crop disease control.

Post-harvest, plant losses can reach up to 50 % of total production, due to several biotic and abiotic factors, including opportunistic microbes (Marcet-Houben et al., 2012). Currently, antibiotics and synthetic fungicides for postharvest decay are restricted due to toxicity, environment impact, resistance and low consumer acceptance (Marcet-Houben et al., 2012). AMPs have been proposed as a solution. (Muñoz et al., 2007; Badosa et al., 2009; Wang et al., 2018; Liu et al., 2019; Lima et al., 2021). PAF56 (GHRKKWFW) prevents rotting of *Citrus sinensis* (L.) Osbeck fruit post-harvest (Wang et al., 2018) and inhibits *Penicillium digitatum*, *Penicillium italicum*, and *Geotrichum candidum* by reducing conidial growth and survival. An *in vivo* study showed peptide's capacity to control orange infection by three phytopathogenic filamentous fungi (Wang et al., 2018). BP15, an undecapeptide composed of cecropin A and melitin, inhibited *Stemphylium vesicarium* conidial germination, growth, and sporulation, as well as demonstrating post-infection antifungal activity on pear leaves (Puig et al., 2014).

Recently, peptide companies have emerged to drive sustainable agriculture. Invaio Sciences conducts research on peptides for citrus greening diseases (https://www.invaio.com/). Micropep (https://micro-pep.com/) develops peptide-based fungicides and herbicides for weed control. Vestaron (https://www.vestaron.com/) has created sustainable peptide-based insecticides that are safe for humans, pollinators, and the environment. These bioinsecticides are designed to target and disrupt the nicotinic acetylcholine receptor in insects. Vestaron has three market-ready peptide products, including Spear®-T Liquid Concentrate,

Table 4
Comprehensive summary of the application of AMPs in agriculture. This table points out target plants, pathogens, and the beneficial impact of AMPs.

AMP	Target plant	Pathogen	Beneficial effects	Ref.
alfAFP	Transgenic potato	*Verticillium dahliae*	Increased disease resistance against *V. dahliae*	(Gao et al., 2000)
Pn-AMP2	Transgenic tobacco	*Phytophthora parasitica*	Enhanced resistance against black shank disease	(Koo et al., 2002)
CEMA	Transgenic tobacco	*Fusarium solani*	Resistance against *F. solani*	(Yevtushenko et al., 2005)
MSI-99	Tomato	*Pseudomonas syringae*	Significantly less development of symptoms of the disease	(Alan et al., 2004)
MSI-99	Banana or Transgenic tobacco	*Fusarium oxysporum*, *Mycosphaerella musicola*, *Sclerotinia sclerotiorum*, *Alternaria alternata* and *Botrytis cinerea*	Greater resistance against the disease compared to the control group	(Chakrabarti et al., 2003)
Mj-AMP1	Tomato	*Alternaria solani*	Enhanced resistance to early blight disease	(Schaefer et al., 2005)
SB-37 and Shiva-1	Transgenic tobacco	*Pseudomonas solanacearum*	Significantly less development of symptoms of the disease and reduced mortality	(Jaynes et al., 1993)
cecropin P1	Tobacco	*Pseudomonas syringae, Pseudomonas marginata,* and *Erwinia carotovora*	Enhanced resistance to phytopathogenic bacteria	(Zakharchenko et al., 2005)
PV5	Tobacco	Tobacco mosaic virus, *E. carotovora, Botrytis cineria, Verticillium sp.* and *F. oxysporum*	Resistance against viruses, bacteria and fungi.	(Bhargava et al., 2007)
pep11	Transgenic tomato	*Phytophthora infestans*	Reduction in lesion size of at least 50 %	(Jones et al., 2004)
MsrA3	Potato	*P. infestans* and *E. carotovora*	Strong resistance to late pest and phytopathogens of pink rot	(Osusky et al., 2004)
MsrA2	Transgenic potato	*Alternaria, Cercospora, Fusarium, Phytophthora, Pythium, Rhizoctonia, Verticillium* and *E. carotovora*	High resistance to highly virulent fungal pathogens and the bacterial phytopathogen *E. carotovora*	(Osusky et al., 2005)
MiAMP1	Transgenic tobacco	*Leptosphaeria maculans*	Control of blackleg disease	(Kazan et al., 2002)
cecropin A	Rice	*Magnaporthe grisea*	Resistance to rice blast disease	(Coca et al., 2006)
cecropin B	Rice	*Xanthomonas oryzae*	Increased strength suggested by reduced size of lesions	(Sharma et al., 2000)
PAF26 and PAF19	Orange fruit	*Penicillium digitatum* and *Penicillium italicum*	Decrease in the percentage of infected wounds in fruit deterioration after harvest	(López-García, 2003)
BP15	Pear fruit	*Stemphylium vesicarium*	Antifungal post-infection activity	(Puig et al., 2016)
Bacillomycin D	Wheat and corn	*Fusarium graminearum*	Significant reduction of virulence and pathogenicity	(Gu et al., 2017)
NoPv1	Grapes	*Plasmopara viticola*	Prevention of *P. viticola* germ tube formation and leaf infection	(Colombo et al., 2020)

Spear®-Lep, and Leprotec®.

3.4. The impact of AMPs on animal husbandry

Antibiotics used for livestock production promote growth and prevent disease but select for resistant pathogens (He et al., 2020). By 2030, antibiotic use in farming may exceed 100,000 tons per year (Van Boeckel et al., 2015). AMPs offer antimicrobial action against several causative agents of livestock disease (Mutwiri et al., 2000; Taha-Abdelaziz et al., 2013; Arayan et al., 2018; Blower et al., 2018; Dassanayake et al., 2018; Cirone et al., 2020). Peptides as feed additives support immunity, provide antimicrobial protection, and regulate gut microbiota, increasing livestock growth, nutrient uptake, and quality of dairy products (Fig. 4). *S. aureus* causes mastitis in the dairy castle leading to decreased milk production, poor quality, and financial losses (Halasa et al., 2007). Two AMPs, NZ2114 and MP1102 reduced *S. aureus* E48 colony forming units (CFUs) isolated from infected bovine milk and mammary epithelial cells without affecting mammalian cell viability (Li et al., 2017). Additionally, the study also investigated the *in vivo* potential of AMPs in *S. aureus*-induced mastitis in mice. Both NZ2114 and MP1102 reduced bacterial load in mammary glands using preclinical models.

Bovine respiratory disease (BRD) is the leading cause of economic loss in the beef industry. *Mannheimia haemolytica, Histophilus somni, Mycoplasma bovis*, and *Pasteurella multocida* are the major bacteria species that cause BRD (Taylor et al., 2010). Several studies show AMPs inhibit these bacterial pathogens (Mutwiri et al., 2000; Arayan et al., 2018; Blower et al., 2018; Dassanayake et al., 2018; Cirone et al., 2020). Four peptide analogues of NK-lysine showed bactericidal activity against *H. somni* at micromolar concentrations (10–30 μM). Fluorescence microscopy showed these AMPs disrupt *H. somni* membrane integrity and induce cell leakage (Dassanayake et al., 2017). Additionally, the tracheal antimicrobial peptide (TAP), a 38-mer β-defensine, was tested against the same pathogens. TAP had antibacterial activity against *M. haemolytica, H. somni,* and *P. multocida* with a MIC in the range of 1.56–6.25 μg/mL (Taha-Abdelaziz et al., 2013). An *in vivo* model was also established for *M. haemolytica* infections in calves, however, TAP did not confer protection, potentially due to physiological factors, such as salt or serum concentrations (Vulikh et al., 2019). The design of robust bioactivity assays that assess AMP stability under physiological conditions is essential to improve early screening and development of hit-to-lead candidates, while also reducing animal use in preclinical studies, aligning with the 3 R's principles – *i.e.*, reduction, replacement, refinement (Mendes et al., 2024).

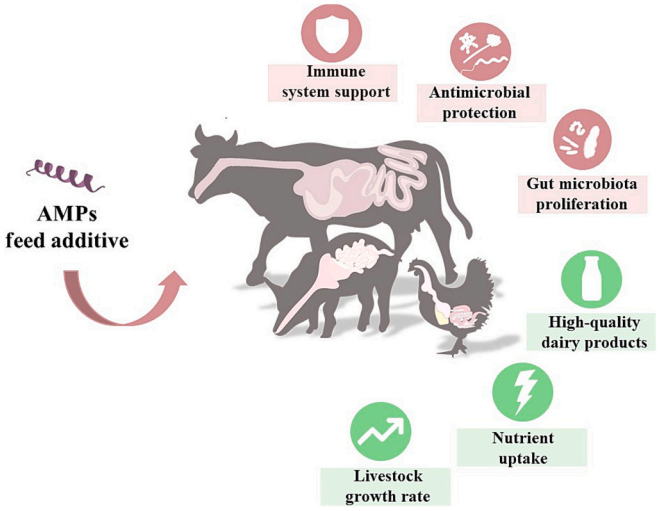

Fig. 4. Positive impacts of AMPs in animal husbandry. These molecules modulate the immune response, gut microbiome, and antimicrobial defence, leading to high-quality dairy products, improved livestock growth, and improved nutrient uptake.

AMP feed additives provide a range of benefits for livestock

including enhanced growth, reproduction, nutrient digestibility, intestinal microflora, intestinal morphology, and immune function (Table 5) (Xiao et al., 2015; Wang et al., 2016b). Lactoferrin is a AMP investigated as a feed additive for pigs (Wang et al., 2006; Wang et al., 2007; Wang et al., 2008b; Tang et al., 2009). Piglets' diets were supplemented with bovine lactoferrin (bLF) treatment (1250 or 2500 mg/kg), which improved the average daily gain (ADG), increased average daily food intake (ADFI) and reduced diarrhea in piglets (Wang et al., 2008b). Another study showed addition of lactoferrin (1000 mg/kg) in weanling pig diet improved growth performance in terms of ADG and ADFI and reduced diarrheal and the presence of intestinal pathogens like *E. coli* and *Salmonella sp.* (Wang et al., 2007). In addition, cipB-LFC-LFA (a hybrid peptide of cipB protein and two lactoferrin fragments) treatment (100 mg/kg) of weaned piglet enhanced growth performance, immune system, and intestinal health (Tang et al., 2009).

The effects of dietary supplementation with AMPs on broiler diets have also been evaluated (Wen and He, 2012; Choi et al., 2013; Xie et al., 2020; Dai et al., 2021). AMP-A3 treatment (90 mg/kg) enhanced the growth performance, and intestinal morphology of broilers, and reduced pathogenic bacteria present in the intestinal microflora (Choi et al., 2013). A combinatorial study tested two AMPs, plectasin, and cecropin, on feeding broilers, which again, improved growth and survival rates (Xie et al., 2020).

Peptidus Biotech (https://peptidus.com/) is a peptide-focused company dedicated to transforming drug discovery for animal health through artificial intelligence-powered peptide design. Its drug discovery pipeline involves identifying lead peptides, optimizing their bioactivity using AI-approaches, and conducting subsequent *in vitro* and *in vivo* analysis. Indeed, this vision has led to the development of MastPept, designed for the treatment of bovine mastitis.

The *in vitro* efficacy of AMPs against livestock pathogens have inspired *in vivo* exploratory studies as feed additives or therapeutics to control diseases. However, peptide stability in high salt concentrations or proteolytic enzymes remains a specific challenge for their wider use in this area (Xu et al., 2023). While questions remain, the benefits of AMPs in animal husbandry are illustrated in a selection of animal models and pathogens. Emerging tools such as genetic editing and AI methods will accelerate development and translation.

Table 5
Implementation of AMPs for enhancing animal health in husbandry. Examples of AMPs used in animal husbandry with details of livestock types and reported effects. Weaning pigs have served as the primary model in research studies.

AMP	Animal	Beneficial effects	Ref.
Lactoferrin	Weanling pigs	Improved growth, reduction in diarrhea and pathogenic bacteria in intestinal microflora	(Wang et al., 2007)
A3	Weanling pigs	Enhanced growth, reduction in coliforms and *Clostridium sp.* in ileum, cecum and feces	(Yoon et al., 2012)
A3	Broilers	Improved growth and intestinal morphology, reduction in pathogenic bacteria in feces	(Choi et al., 2013)
P5	Weanling pigs	Improved growth and digestibility of nutrients, reduction in coliforms in feces and intestine	(Yoon et al., 2013)
Cecropin AD	Weanling pigs	Decreased diarrhea incidence on pigs challenged with *E. coli,* reduced total viable counts of cecal *E. coli*, increased *Lactobacilli* counts in cecum	(Wu et al., 2012)
cipB-LFC-LFA	Weanling pigs	Modulation of the immune system, improved growth and intestinal health	(Tang et al., 2009)
Colicin E1	Weanling pigs	Improved growth, reduced incidence and severity of postweaning diarrhea	(Cutler et al., 2007)
Bovine lactoferrin	Weanling pigs	Improved growth, reduced diarrhea	(Wang et al., 2008b)
Cecropin A-D-Asn	Broilers	Improved growth and intestinal health	(Wen and He, 2012)
Plectasin-derived peptides	Mouse model of bovine mastitis	Reduced *Streptococcus aureus* load and concentrations of proinflammatory cytokines TNF-α and IL-6 in mammary glands	(Li et al., 2017)
Sublancin	Broilers	Reduced cecal *Clostridium perfringens* counts and concentration of cytokines IL-1β, IL-6, TNF-α	(Wang et al., 2015)

4. Challenges associated with the use of AMPs

So far, we have discussed the diverse potential applications of AMPs across various markets. Nonetheless, several drawbacks must be considered, even in the initial development phases. AMPs can exhibit instability under physiological conditions. Factors such as high salts concentrations, temperature, pH, and proteolytic enzymes can influence the efficacy of AMPs (Alves et al., 2024). Certain food products contain high salt concentrations (Albarracín et al., 2011) or the presence of monovalent and divalent cations (Na^{+}, Ca^{2+}, Mg^{2+}) may interfere with the interaction between AMPs and the negatively-charged membrane of bacteria, thereby reducing the bioactivity of AMPs (Zhao et al., 2022). The bioactivity of several members of cruzioseptins was diminished in the presence of various salt concentrations (NaCl 150-300 mM, $MgCl_2$ 1 mM, and $CaCl_2$ 8 μM) (Valdivieso-Rivera et al., 2022). Moreover, the biological activity of AMPs must be preserved across a broad range of pH, including acidic conditions, for certain biotechnological applications (Wang et al., 2022). Some AMPs also lose their activity in acidic environments due to pH-induced conformational changes (Mardirossian et al., 2021).

AMP proteolytic degradation is a significant challenge for applications like feed additives. Linear AMPs, in particular, are highly susceptible to a wide variety of proteases found in the digestive processes, serum and those produced by pathogenic microorganisms (Lai et al., 2022). Each proteolytic enzyme exhibits specific recognition patterns and is capable of cleaving peptide bonds (Jamal et al., 2025). For instance, arginine and lysine residues are substrates of trypsin-like proteases, which are found in the gut of several animals (Ferguson et al., 2022). The protection of both basic amino acids is crucial due to their role in the MOA of AMPs. Moreover, cytotoxicity also represents a notable challenge to the biotechnological use of AMPs (Somase et al., 2024). A common approach to evaluate AMP-induced toxicity is the traditional absorbance-based hemolysis assay, which measures the damage of AMPs caused to red blood cells (Robles-Loaiza et al., 2022). Several studies have shown that certain AMPs lack selectivity, affecting both microbial and host cells (Enninful et al., 2024). Therefore, this drawback undermines their safety and effectiveness. This review specifically highlights key limitations of AMPs (Fig. 5), as well as outlining potential strategies to address them. For further details, we recommend referring to the following articles (Cardoso et al., 2021; Chen et al., 2022; Xu et al., 2023).

5. Existing and emerging technologies to produce AMPs

Delivering AMPs for the mass market is another challenge that requires multidisciplinary collaboration to advance the scale-up of peptide therapeutics for wider industrial application. Expanding their use demands cutting-edge and cost-effective and sustainable technologies capable of large-scale production of AMPs and their derivatives. Advancements in peptide therapeutics will require collaborations with diverse research fields including synthetic chemistry, chemical engineering, industrial biotechnology, and synthetic biology. Specifically, we will now critically evaluate the advantages and disadvantages of established (*e.g.*, chemical synthesis, plant-based expression) and emerging methods (*e.g.*, cell-free biosynthesis) for AMP production

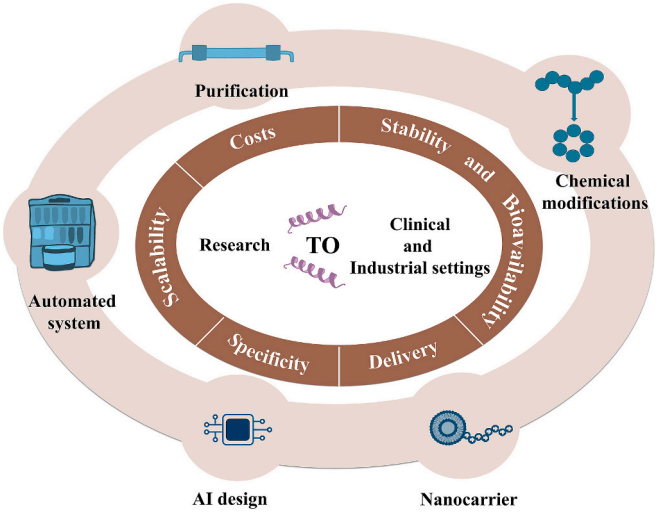

Fig. 5. The bottlenecks in the development of peptide-based products for biotechnological and industrial applications. The roadmap of peptides, from design to clinical and industrial applications, is fraught with challenges, such as cost, specificity, scalability, delivery, stability, and bioavailability. These obstacles have been addressed by multidisciplinary teams leveraging a variety of innovative approaches, including optimization the purification steps, automatization, AI design, nanocarrier, and chemical modification.

(Fig. 6).

5.1. *Chemical-based AMP production*

Chemical synthesis of peptides remains the gold standard for discoveries at the bench level to therapeutic application. Peptide synthesis uses either in solution phase or in solid phase (Andersson et al., 2000). Solution phase synthesis (SPS) strategy, firstly developed by Vincent du Vigneaud in 1953 to produce the polypeptide hormone oxytocin, relies on the selection and construction of peptide fragments, which are then purified and coupled in solution to obtain the complete sequence (Zompra et al., 2009). SPS is typically used to obtain small- and medium-length peptides up to the ton scale (Andersson et al., 2000; Zompra et al., 2009). The key limitation of this synthesis process is time. About 17 commercial peptides are manufactured by SPS, including oxytocin, angiotensin-converting enzyme (ACE) inhibitors, vasopressin analogues (9–12 amino acids), luteinizing hormone–releasing hormone analogues (9–10 amino acids), HIV protease inhibitors, and calcitonins (32 amino acids) (Andersson et al., 2000).

Solid phase peptide synthesis (SPPS), introduced by Merrifield (Merrifield, 1963), is the dominant technology. SPPS involves binding of the amino acid carboxyl group to an insoluble solid support (resin) and the subsequent elongation of the peptide chain by formation of an amide bond between the carboxyl group (-COOH) of the incoming amino acid and the amino group (NH_2-) that previously bonded to the resin. This reaction is repeated until the desired sequence is obtained, before peptide cleavage from the resin (Guzmán et al., 2007). SPPS requires coupling reagents and additives, which influence the efficiency of synthesis. A suitable coupling reaction leads to higher yields, avoiding the phenomenon of racemization. Unlike SPS, there is no need for purification of intermediary products, which leads to an easier, faster, and less

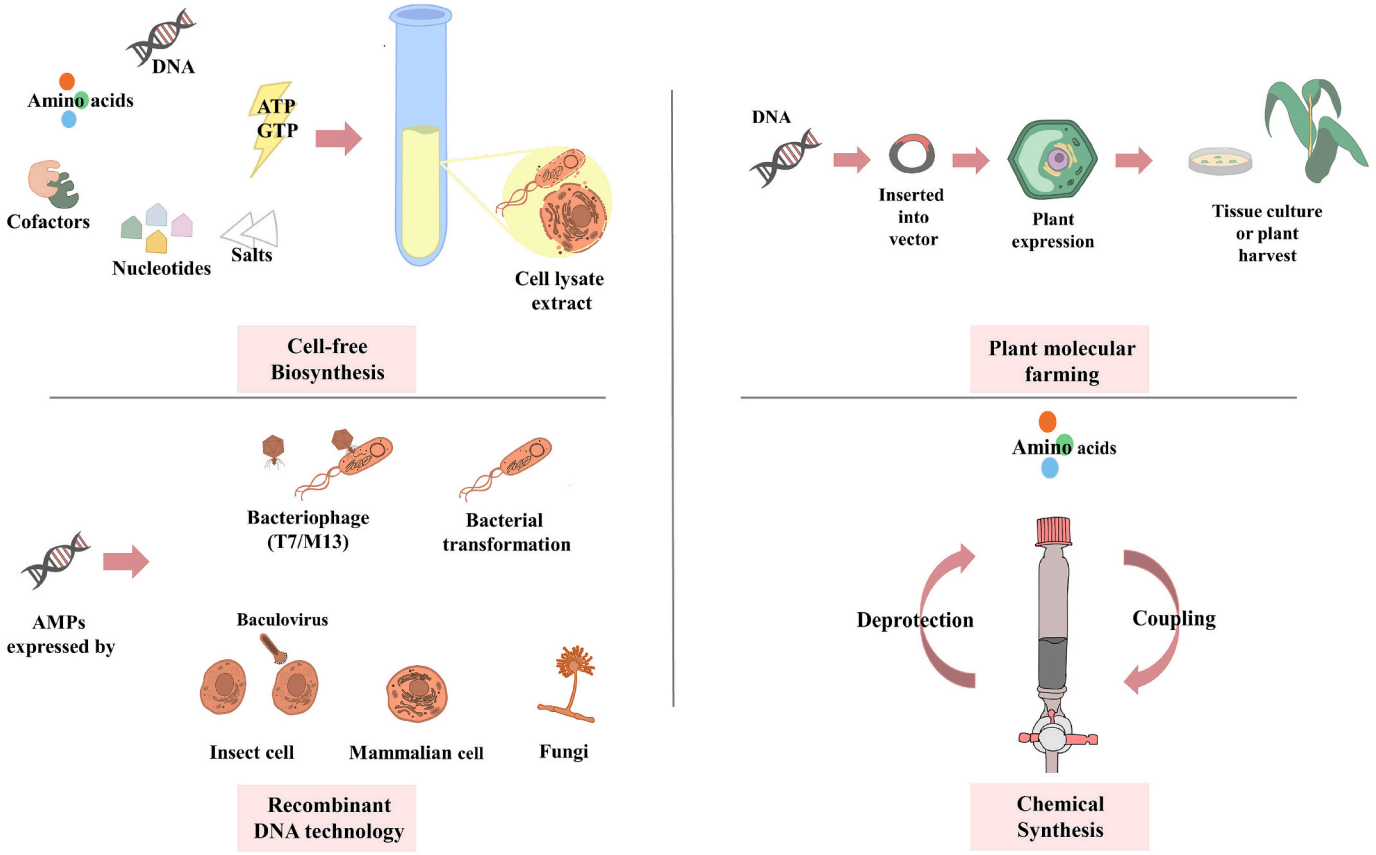

Fig. 6. AMP production platforms. Current modern approaches employed for obtaining AMPs, include chemical synthesis, recombinant technology, cell-free biosynthesis and plant molecular farming. The success of AMP across many industrial sectors calls for the need for advancements in AMP manufacturing.

laborious process (Albericio, 2004; Zompra et al., 2009). The SPPS strategy is capable of synthesizing peptides in a production scale in the range of kilograms. Several commercial peptides have been synthesized with this methodology, including thymalfasin (28 amino acids), thymosin α-1 (28 amino acids), ziconotide (25 amino acids), calcitonins (9–10 amino acids), and luteinizing hormone–releasing hormone analogues (9–10 amino acids) (Andersson et al., 2000; Bruckdorfer et al., 2004). The simplified chemistry has led to automation in manufacturing, and many peptide synthesizers are currently commercially available.

A hybrid approach has been developed using the SPS and SPPS strategies together. In this case, the peptide fragments are prepared by SPPS, and these blocks are coupled in solution to obtain the desired peptide. This approach combines the benefits of SPPS and SPS, including rapid process synthesis. The production of Fuzeon T-20 (36 amino acids), a commercial peptide for the treatment of HIV, is done through the hybrid methodology (Bruckdorfer et al., 2004; Zompra et al., 2009).

5.2. Plant-based AMP production

The use of plants or plant cell cultures as a production platform for proteins of industrial or pharmaceutical importance is called plant molecular farming (PMF) (Obembe et al., 2011; Shanmugaraj et al., 2020). Over 100 pharmaceutical proteins have been expressed and characterized in plants, and 20 PMF-made pharmaceuticals are being evaluated in preclinical and clinical trials, one of which has been approved by the FDA (Yao et al., 2015). PMFs have several advantages, including high product throughput, straightforward manufacturing methods, the ability to perform post-translational modifications, and low production costs compared to other synthesis approaches. In addition, this technology is considered safe because there is reduced risk of contamination of the product by animal or human pathogens (Fischer et al., 2004; Yao et al., 2015; Shanmugaraj et al., 2020).

This approach has also been used to produce several AMPs (Bundó et al., 2014; Shanmugaraj et al., 2021). The recombinant LL-37 peptide was expressed in barley seed with a yield of up to 0.55 mg/kg of grain, and displayed *E. coli* antibacterial activity (Holásková et al., 2018). Protegrin-1, produced in low-alkaloid *Nicotiana tabacum*, was bioactive against *K. pneumoniae*, *S. aureus*, *E. coli*, *Mycobacterium bovis*, and *C. albicans* (Patiño-Rodríguez et al., 2013).

5.3. Bacteriophage-based AMP production

Bacteriophage expression is a powerful tool for peptide production in host cells (Lemon et al., 2019; Masuda et al., 2021; Ludwig et al., 2022). Phage-displayed peptides offer benefits such as high specificity, cost-effective production, high purity and easy scalability (Wang et al., 2024). Lemon et al. (2019) inserted the coding sequence for AMP 1018 into the T7 phage genome. 1018 AMP, expressed by the T7 bacteriophage, showed antibacterial and antibiofilm activities. In addition, liquid chromatography-tandem mass spectrometry (LC-MS/MS) detected AMP 1018 at a concentration of 300 ng/L. Similarly, lacticin Q, a bacteriocin produced by *Lactococcus lactis* QU5, was also integrated into the T7 bacteriophage genome. The recombinant lacticin Q inhibited the bacterial growth of *E. coli* and *Bacillus coagulans* (Masuda et al., 2021). The LC-MS/MS analysis did not allow the detection of recombinant lacticin Q in the samples. The researchers proposed two possible reasons, namely (1) binding of the AMP with negatively charged cell membrane or (2) limited synthesis of lacticin Q by the engineered phage (Masuda et al., 2021). Wang et al. (2024) constructed and screened several phage-displayed peptides against Gram-positive and Gram-negative bacteria. All of them inhibited specifically the growth of *S. aureus*. They also evaluated cytotoxicity against red blood cells and HEK293T and the results demonstrated these peptides do not induce toxicity. Additionally, *in vivo* experiments were conducted to evaluate the biosafety and therapeutic effect in mice and piglets. Group treated with phage-displayed peptides showed low levels of bacterial load compared to the control group and the treatment did not lead to undesired effects.

5.4. Bacterial-based AMP production

Bacterial expression of AMPs provides significant advantages including low-cost media, fast replication, strong gene expression tools, and high yields of recombinant protein production (Deng et al., 2017; Sampaio de Oliveira et al., 2020). However, there are challenges that must be overcome to achieve effective production of AMPs in bacteria. These include the potential for AMP toxicity to the host as well as issues related to their instability; linear AMPs are prone to proteolytic degradation. In addition, the sequence diversity of peptides, post-translational modifications and glycosylation increase the complexity of obtaining AMPs in this type of production system (Li, 2011). The bacterial systems most used as hosts for AMP production are *E. coli, B. subtilis,* and *Lactococcus lactis* (Sampaio de Oliveira et al., 2020; Neef et al., 2021; Yang et al., 2021).

E. coli is a widely used host for recombinant protein production (Deo et al., 2022), including AMPs. There are well-established protocols and extensive knowledge of its genetics, biochemistry and physiology. Commercial *E. coli* strains used for AMP production include BL21 (DE3), Origami (DE3), and Rosetta (DE3) (Wibowo and Zhao, 2019). For example, fowlicidin-2, an AMP from chicken, was expressed in *E. coli* BL21 (DE3) with a yield of 6 mg/L (Feng et al., 2015). Studies have also highlighted the low-cost synthesis and large-scale production of AMPs (Zhao et al., 2015; Deo et al., 2022). Gaglione et al. (2019) assessed a cost-effective approach for producing AMPs in *E. coli*, focusing on the development of an enhanced culture medium that supports high cell densities and expression levels. When the process was scaled up from 100 mg per batch to 1000 mg per batch (5 L fermenter) this lowered the cost from 253 €/mg to 42 €/mg. A key limitation of *E. coli* expression is challenges with post-translational modification, peptide solubility, and contamination with endotoxins, which is an issue for *in vitro* and *in vivo* assays (İncir and Kaplan, 2024).

5.5. Fungal-based AMP production

Fungi are powerful cell factories for engineering the production of AMPs (Lübeck and Lübeck, 2022). Particularly, filamentous fungi and yeasts offer many advantages as expression systems including high yields of protein production, cost-effective media, and post-translational modification enzymatic machinery (Meng et al., 2017). Also, *Aspergillus* and *Penicillium* genera are considered GRAS by the FDA (Garvey, 2022; Lübeck and Lübeck, 2022). Focusing on unicellular yeasts, the predominant species for recombinant protein production are the baker's yeast *S. cerevisiae* and the methylotrophic yeast *Komagataella pastoris* (formerly *Pichia pastoris*), which have well characterized physiology, biochemistry and genetics (Baghban et al., 2019).

K. pastoris is an excellent host for secreting recombinant proteins and short peptides in high concentrations and purity into the medium, thereby minimizing both the time and cost involved in the purification process (Cao et al., 2018). For example, apidaecin Ia was expressed with *K. pastoris* as a fusion protein and was active after proteolytic release (Cao et al., 2018). Similarly, *K. pastoris*-based expression of the LL37 peptide reached 154.8 mg/L after 96 h of fermentation (Zhan et al., 2021). Related work has also optimized the production of AMPs with *K. pastoris* using low-cost culture media producing up to 105 μg/L crude melittin in a minimal methanol medium within 5 days at a total of $10 USD (Moridi et al., 2020).

5.6. Insect cell-based AMP production

Insect cells are also strong hosts for AMP production. Recombinant AMPs can be expressed using two methods: stable expression via

genomic integration of the AMP-encoding gene or baculovirus gene expression (Carballar-Lejarazú et al., 2008; Almasia et al., 2017; Zitzmann et al., 2017; Käßer et al., 2022). Insect-based expression provides post-translational modifications that may be necessary for the structural and functional characteristics of some AMPs (Zitzmann et al., 2017). Other benefits of this technology include the rapid production of large amounts of protein, its cost-effectiveness in comparison to mammalian cell-based methods, and its ability to produce toxic peptides (Rivera-de-Torre et al., 2022). Several AMPs have been synthesized using insect cells. For instance, *Anopheles gambiae* was used to express scorpine, an AMP isolated from the venom of the scorpion *Pandinus imperator* (Carballar-Lejarazú et al., 2008). Recombinant scorpine displayed antimicrobial, antiparasitic, and antiviral properties (Carballar-Lejarazú et al., 2008). Snakin-1, an AMP derived from *S. tuberosum*, was expressed from *Spodoptera frugiperda* cells, providing soluble peptide with a yield of 6.3 mg/L. (Almasia et al., 2017).

5.7. Mammalian cell-based AMP production

While high-cost, mammalian cell line-based production systems provide enhanced protein folding, glycosylation and post-translational modifications, and typically used to make complex recombinant proteins and complexes (Wurm, 2004). Chinese hamster ovary cells (CHO) are the main mammalian cell line used for recombinant protein production due to their ease of use and safety profile. In addition, murine myeloma cells (NS0 and Sp2/0), hamster kidney (BHK), human embryonic kidney (HEK-293) and human retinal cell line PER-C6 are also used to produce recombinant proteins (Kim et al., 2012; Zhu, 2012; Liu et al., 2017a). However, there are limited studies of using these expression systems to produce AMPs. One example, is production of bacteriocin enterocin P with CHO cells and characterization of its bioactivity against Gram-negative and Gram-positive bacteria, including multidrug resistant bacteria (Tanhaeian et al., 2019).

5.8. Cell-free-based AMP production

Cell-free protein synthesis (CFPS) is an emerging method for rapid synthesis. Its greatest advantage is making AMP derivatives. As a platform technology within synthetic biology, CFPS is attracting significant interest throughout academia and industry for a range of applications (Silverman et al., 2020) – *e.g.*, protein production, biosensors, metabolic engineering. CFPS requires a cell extract, DNA/RNA template and an energy solution to catalyze peptide or protein synthesis. CFPS shares similarities to synthetic chemistry approaches but instead uses ribosomes to catalyze peptide extension. Using *E. coli* CFPS as the main model, the ribosomes are highly efficient and fast molecular machines that catalyze polypeptide synthesis at rates of ~2–5 aa/s, although this is slower than inside a cell (10–20 aa/s) (Dennis and Bremer, 1974; Dennis and Nomura, 1974; Young and Bremer, 1976). Translation elongation efficiency is optimized to close to 100 % (Szavits-Nossan and Ciandrini, 2020), with incomplete translation products and mRNA transcripts degraded and recycled into amino acids and nucleotides, respectively (Inada, 2020). A prokaryotic cell extract contains RNA, metabolites and about 1000 to 2000 proteins derived from a cell that is typically harvested and is lysed at the point of exponential growth (Foshag et al., 2018; Chengan et al., 2023; Rasor et al., 2023). Key proteins include the core transcriptional and translational enzymatic machinery, while there are several important classes of proteins, including metabolic enzymes that help regenerate energy (Kim and Swartz, 2000; Kim and Swartz, 2001).

Making AMPs with CFPS provides advantages and disadvantages. First a cell extract is derived from a cell, where the cell-membrane, cell-wall and genomic DNA is removed. While these barriers are essential to a cell, they are also targets for AMPs, which can be toxic during prokaryotic or eukaryotic cell-based expression (Benfield and Henriques, 2020). Genomic DNA along with its associated regulatory DNA binding proteins, also acts as a sensor of stress and toxic molecules. The removal of the genomic DNA enables the production of toxic biomolecules, such as AMPs. The lack of a cell wall barrier also allows fast purification, real-time monitoring, rapid sampling, as well as direct manipulation of peptide synthesis (Garenne et al., 2021). A key strength of CFPS is the rapid and microscale engineering of AMPs within 24 h cycles. A DNA template sequence is easy to diversify the encoding peptide (*i.e.*, by PCR, mutagenesis libraries), while modification of the amino acids (Fleming et al., 2019; Liu et al., 2020a; Liu et al., 2024; Si et al., 2021) and non-canonical amino acid incorporation is possible (Seki et al., 2018; Stech et al., 2021; Ranji Charna et al., 2022). Traditional gene-cloning steps can be avoided by using linear DNA templates (Khambhati et al., 2019). This reduces the timeframe for peptide synthesis and derivatization, and with the aid of automation (Moore et al., 2018; Garamella et al., 2019; Hunt et al., 2023), has the potential to develop a pipeline for rapid synthesis of AMP analogues and biological evaluation. In addition, CFPS was used to evolve membrane-targeting AMPs using ribosome display (Xie et al., 2006).

There are also challenges in making AMPs with CFPS. Apart from scale and cost, a major consideration for making AMPs with an *E. coli* cell extract based CFPS reaction is peptidases and proteases. The N-terminal amino acid in prokaryotic protein synthesis is formyl-L-methionine (fMet). After protein synthesis, many nascent proteins undergo deformylation (Meinnel and Blanquet, 1993), before cleavage by a methionine aminopeptidase. This activity is enhanced if the second amino acid is small and uncharged (Ben-Bassat et al., 1987). The primary sequence of the peptide is an important consideration for designing and engineering AMPs with prokaryotic CFPS. However, a potential solution to this problem is the use of eukaryotic CFPS, although commercial kits are more expensive (Harbers, 2014; Buntru et al., 2022).

In prokaryotic CFPS, peptidases and proteases present in the cell extract have a broad range of mechanisms, recognition specificities, and cellular location. There are at least 74 peptidases/proteases encoded by the *E. coli* MG1655 K-12 genome, many of them are essential genes. Peptidases and proteases are required for cell growth, through maturation of integral proteins, or repair and recycling of mis-folded or mis-translated proteins. To overcome this issue, most cell-free studies have used the Protein synthesis Using Recombinant Elements (PURE) system, which has all the purified *E. coli* translation factors, ribosomes and T7 RNA polymerase to catalyze gene expression (Shimizu et al., 2005; Shimizu and Ueda, 2010). For instance, (Pandi et al., 2023) developed a pipeline for the rapid and production of AMPs using cell-free biosynthesis based on the *E. coli* PURE system and linear DNA templates. Their results demonstrated the potential of CFPS for low-cost synthesis of AMPs and the antibacterial characterization within less than 24 h. Four lactoferricins were expressed in a CFPS system based on RTS 100 *E. coli* HY kit at concentrations in the range of 1.25–10.0 μg/mL, showing significant antimicrobial activity by the microdilution assay (El-Baky et al., 2021). A new CFPS platform based on lysates derived from the fast-growing bacterium *Vibrio natriegens* was recently developed (Des Soye et al., 2018). The system was used to synthesize the AMPs cecropin A, cecropin P1, and opistoporin. In the case of opistorin, the expression exceeded 250 μg/mL.

Examples of AMPs produced by *E. coli* CFPS, include the human beta-defensin-2 (hBD2), a small cationic peptide with broad range of antimicrobial activity. hBD2 was fused to an N-terminal green fluorescence protein (GFP) to enhance synthesis (0.35 mg/mL) and limit protease degradation (Xu et al., 2005). To release the active hBD2 peptide, an enterokinase protease site was placed between the GFP and AMP. The authors also used a semi-continuous dialysis reaction to maximise the yields of GFP-hBD2 up to 2 mg/mL. In addition, because hBD2 was fused at the C-terminus, the presence of rare codons in the gene did not limit protein synthesis (Xu et al., 2005). In another study, an *E. coli*-derived CFPS system was used to synthesize several AMPs, among which, cecropin B exhibited the highest yield (188–219 ng/μL) at a cost of $

3.10–3.60 USD per μg (Pardee et al., 2016). The costs of AMP production depend on several aspects, including the use of amino acids, DNA, co-factors, and the substrates selected as an energy source. A few studies have evaluated cheaper and more efficient cell-free methods for protein synthesis (Calhoun and Swartz, 2005; Zemella et al., 2015; Khambhati et al., 2019; Nagappa et al., 2022).

5.9. A comparative perspective on the technologies utilized in peptide synthesis

Selecting the best method for peptide production depends on several factors, including the intended application, cost, scalability, and technical requirements. Hence, given the importance of obtaining enough peptides for translational applications, we compare the various approaches available for peptide production (Fig. 7). Chemical synthesis methods are valuable for producing high-purity and short peptide sequences but may be limited by higher costs and restricted scalability. Alternatively, DNA-recombinant technology is highly versatile, enabling the production of complex peptides and scalable solutions, but it often requires extensive expertise and compliance with regulatory standards, which can increase costs. For instance, plant-based expression is a more sustainable, cost-effective strategy, and scalable, although there are challenges in downstream processing. Cell-free biosynthesis offers a flexible and rapid alternative to conventional methods, its key strengths are in efficiency and derivatization, its limitations include scalability, cost, and peptide degradation.

In summary, peptide synthesis technologies are rapidly evolving, with chemical methods (*e.g.*, SPPS) already at market, while biological recombinant methods are at different levels of technology readiness for advancement into commercial exploitation. Future research will focus on optimization of cost and scalability or exploiting specific advantages such as derivatization or post-translational modification. Here, interactions between academia and industry will increase commercialization opportunities in peptide biotherapeutics, while there is also potential for hybrid chemistry and biological approaches to provide key limitations.

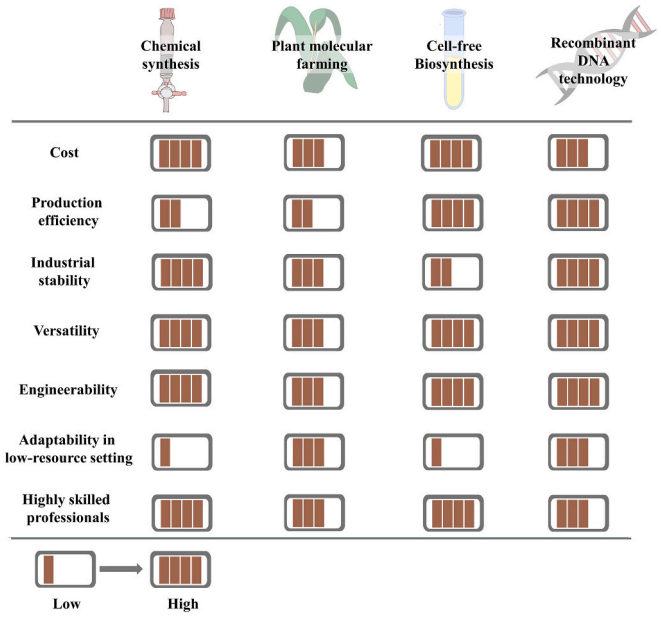

Fig. 7. Strategies for peptide production: a side-by-side comparison. The bars provide a visual representation of the theoretical favorability of each parameter, where several bars indicate greater potential. This analysis is derived from the authors' professional expertise, extensive literature review, though it inherently involves a degree of subjective evaluation. Cost refers to the financial investment required to produce peptide. Production efficiency describes how effectively a production process converts inputs (such as raw materials, energy, labor, and time) into desired peptides. Industrial scalability involves adapting production methods to meet the demands of industrial and biotechnological applications. Versatility denotes to the ability of peptide production methods to be applied across diverse applications and settings. Engineerability describes the ease with which peptide production strategies can be optimized or tailored. The adaptability to low-resource settings addresses the feasibility of using a production method in environments with limited resources or infrastructure. On the other hand, demand for skilled professionals assess the level of expertise required to execute a production method efficiently.

6. The global market for peptide-based products

The peptide industry is rapidly growing. The market is varied, offering a range of potential products and applications for health and agricultural industries. The market for peptide-based drugs surpassed US$ 70 billion in sales in 2019 (Anand et al., 2023). However, a small number of medicinal peptide products are responsible for most global sales. Insulin and its analogues produced a total revenue of US$ 25 billion. Dulaglutide (Trulicity), used to treat diabetes, was the second best-selling drug with US$ 4.39 billion in sales. In the oncology drug market, sales of leuprolide and octreotide achieved US$ 2.02 and US$ 1.59 billion, respectively. Novo Nordisk, Eli Lilly, Sanofi, Dainippon Sumitomo, AbbVie, Astellas, and Takeda are among the major companies selling peptide drugs (Muttenthaler et al., 2021). In the last five years, 14 peptide-based drugs were approved by the FDA to treat diabetes type (II), obesity, chronic idiopathic constipation, hypoactive sexual desire disorder, among others (Al Musaimi et al., 2021). To date, a total of 105 peptides have been authorized by the FDA and European Medicines Agency (EMA) for therapeutic or diagnostic use, over 500 peptides are being tested in preclinical studies, and another 150 are in clinical trials (D'Aloisio et al., 2021).

Regarding the AMP market, this is in the early stages. In 2015, this market was worth US$ 1.06 billion with more and more companies emerging (Table 6). From 2017 to 2030, the AMP market is expected to expand at a compound annual growth rate (CAGR) of 7.5 % (Kazemzadeh-Narbat et al., 2021). Also, the nisin market is expanding quickly in the Asia-Pacific region, then in Europe and North America. The global market was valued at US$ 545.5 million by 2020 and is predicted to increase to US$ 553 million USD by 2025, with a CAGR of 4.5 %. Some of the leading companies in nisin production include Danisco A/S (Copenhagen, Denmark), DSM (Heerlen, Netherlands), Sivelee B.V. (Netherlands), Galactic (Escanaffles, Belgium), Shandong Freda Biotechnology Co., Ltd. (Jinan, China), Zhejiang Silver-Elephant Bio-engineering Co., Ltd. (Taizhou, China), and Chihon Biotechnology Co., Ltd. (Luoyang, China) (Juturu and Wu, 2018). In addition, several peptides are currently being marketed as biopesticides. At present, the global biopesticide market is valued at US $3 billion and is expected to experience a compound annual growth rate (CAGR) of over 15 % (Damalas and Koutroubas, 2018).

7. Concluding remarks

The emergence of antibiotic-resistant pathogens is a transnational issue that transcends the borders of the medical clinic, with drastic consequences and environmental concerns in several sectors, such as agriculture, food security and animal husbandry. Looking ahead, AMPs are likely to play an important role and offer a multifaceted approach to address these global challenges. Over the last decade, the notion of antibiotic resistance has motivated the development of biopharmaceutical products. However, we focus on evaluating the biotechnological applications of AMPs specifically for agriculture, food preservation, aquaculture, and animal husbandry. In agriculture, AMPs hold promises for sustainable pest and disease management, offering natural solutions to reduce reliance on chemical pesticides and antibiotics. Moreover, their potential as growth promoters and immunostimulants, in animal husbandry and aquaculture, underscore their importance in enhancing

Table 6
A non-exhaustive list of AMP-producing companies. AMPs have driven significant scientific discoveries and contributed to the emergence of companies in several countries. Most of these companies are emerging from developed countries, with fewer exceptions from developing countries.

Name	Country	Year of foundation	Discovery/Development	Link
Lytix Biopharma AS	Norway	2003	AMP-based drugs for the treatment of cancer	https://www.lytixbiopharma.com/
Madam Therapeutics	Netherlands	2011	AMPs for tackling antimicrobial resistance	https://madamtherapeutics.com/
Dermagen AB	Sweden	2004	AMPs for the treatment of bacterial and fungal skin infections	Unavailable
I-Corps	United States	2012	Use of AMPs as additives for animal feed	https://grantome.com/grant/NSF/IIP-1262212
Peptilogics	United States	2013	Use of a computational platform for the development of peptides against multiresistant microorganisms	https://peptilogics.com/
Riptide Bioscience	United States	2014	AMPs-based drugs	http://www.riptidebio.com/
BioAMPS International LLC	United States	2009	AMPs for the treatment of systemic infectious diseases	Unavailable
ContraFect Corporation	United States	2008	Discover and development of AMPs and lysins for treating drug-resistant infectious diseases	Unavailable
Allvivo Vascular, Inc.	United States	2000	AMPs-based antimicrobial technologies for preventing biofilm in medical devices	https://allvivo.com/
CSA BioTech	United States	2012	Use of small AMP-mimicking molecules to treat diabetic foot ulcers, pressure ulcers, epidermolysis bullosa, onychomycosis	http://csabiotech.com/
Diapin Therapeutics	United States	2011	A three amino acid peptide for the treatment of Type 2 diabetes	https://www.diapin.com/
Vestaron	United States	2005	Development and marketing of peptide-based biopesticides	https://www.vestaron.com/
AMP Therapeutics GmbH	Germany	2009	AMPs for the treatment of drug-resistant infections	Unavailable
Numaferm	Germany	2017	Recombinant expression of peptides, pepteins and proteins	https://numaferm.com/
Invaio	United Kingdom	2017	Developing AMPs for crop protection	https://www.invaio.com/
Amprologix	United Kingdom	2018	Use of artificial intelligence to identify and develop new AMP-based antibiotics	https://www.amprologix.com/
Antimicrobial Peptide Biotechnologies S.L (AMPbiotech)	Spain	2009	Synthetic AMPs for agriculture, food, veterinary, pharmaceuticals (new antibiotics), and other sectors like cosmetics, biomaterials or medical implants.	http://www.ampbiotech.com/
SetLance	Italy	Unknown	Novel peptide-based drug candidates for the treatment of bacterial infections and cancer	https://setlance.com/
Nuritas	Ireland	2014	Novel and active peptides with health benefits	Unavailable
Omnix Medical	Israel	2015	AMPs for the treatment of infectious diseases	https://omnixmedical.com/
PharmaHolding	Norway	2017	AMP-based drugs for treatment of infectious diseases	https://www.pharmaholdings.no/
Peptidus	Brazil	2021	Use of peptides for cosmetics, animal husbandry and health industries	https://peptidus.com/
Syngulom	Belgium	2013	Synthetic biology startup using bacteriocins to improve microbial fermentation	https://syngulon.com/

food security and environmental sustainability. Whereas AMPs serve as natural antimicrobial agents to inhibit microbial growth, extend food shelf-life, address foodborne illnesses and reduce food waste. The literature continued to be dominated by the therapeutic view of AMPs as antibiotic drugs. However, a progressive shift shows several studies starting to explore AMPs for industrial applications. As more applications are highlighted, the scientific community adds another valuable perspective to the future of peptide science. We conclude that most studies involving AMPs were conducted in *in vitro* conditions. While promising, we caution this preliminary evidence does not necessarily replicate at the *in vivo* level, where further research is required to fully realize the potential for AMPs in addressing global issues. Overall, the biotechnological applications of AMPs represent a compelling frontier and a passport to a viable path forward to fight against antibiotic resistance and the quest for sustainable solutions in agroindustry. Future research should concentrate on identifying and clarifying the specific limitations of AMPs within each industry, with the goal of fostering successful applications. For example, proteolytic degradation may pose a challenge for medical uses, such as intravenous injections. Nonetheless, in sustainable agriculture, proteolysis could be beneficial, as it leads to the breakdown of AMPs into small peptides and amino acids. Similarly, future studies should focus on peptide engineering to enhance their efficacy and selectivity while ensuring cost-effective production.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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