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Bacterial contamination in starch-to-ethanol fermentations: Can bacteriocin-producing *Saccharomyces cerevisiae* offer a solution?

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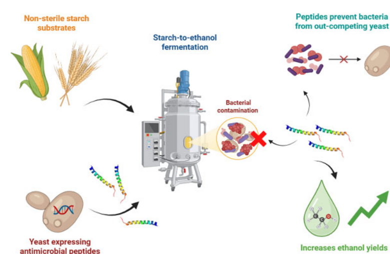
Abstract

Increasing interest in the bioeconomy has spurred the development of integrated methods to convert organic waste streams, particularly starch-rich substrates, into bioethanol. However, starch-based ethanol fermentations are vulnerable to bacterial contamination, particularly by lactic acid bacteria (LAB). Severe contamination can cause significant economic losses due to stuck fermentations and ethanol plant shutdowns. Although bacterial contamination can be managed with antibiotics, this approach is not cost-effective at an industrial scale and may increase the risk of selecting for antibiotic-resistant strains. Natural antimicrobial peptides (AMPs) can inhibit LAB contaminants in yeast fermentations, but commercial applications are limited by their low abundance and high production costs. Engineering *Saccharomyces cerevisiae* to produce recombinant AMPs might provide a cost-effective strategy to control LAB, thereby boosting ethanol yields during fermentation. Despite a comprehensive toolkit for gene expression in *S. cerevisiae*, only a few successful cases of bacteriocin expression have been reported. Since starch-to-ethanol fermentation is a key application for recombinant AMPs, this review explores strategies to optimize the expression of bacteriocin-encoding genes in *S. cerevisiae*. The ideal scenario would be a single yeast strain capable of producing amylases for starch hydrolysis, fermenting glucose to ethanol, and expressing bacteriocins to inhibit LAB contaminants.

One-sentence summary: **Yeast strains can produce heterologous antimicrobial peptides that help prevent contaminating bacteria from interfering with the starch-to-ethanol fermentation process.**

Keywords **bacteriocins, starch-based ethanol, recombinant yeast expression.**

Graphical abstract



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Introduction

Approximately 200 billion litres of biofuels are produced worldwide each year, with the United States accounting for over 38.8% of the global market (Statista, 2024). Bioethanol is blended with petrol to produce "flex-fuel" grades E85, E10, or E5, depending on the bioethanol content. *Saccharomyces cerevisiae* is the preferred organism for bioethanol production due to its rapid growth, saccharolytic properties, ability to thrive on various substrates, pH, water activity, and temperature tolerance, and resistance to ethanol (Azhar et al., 2017; Brexó & Sant'Ana, 2017). Fermentation with selected strains of *S. cerevisiae* can also produce high-value end products in bioethanol refining, making the process more economically sustainable (Sun et al., 2024).

Bioethanol production is classified by feedstock type (Figure 1): first-generation bioethanol is obtained by fermenting edible crops such as sugarcane (molasses), sugar beet, sweet sorghum, maize, barley, rice, cassava, potatoes, and waste products like whey (Cripwell et al., 2020; Zou & Chang, 2022). Dry-milled maize kernels are boiled for a few minutes at 105 °C, cooled to 80–90 °C, and treated with a thermostable α-amylase to hydrolyse the α-1,4 glycosidic bonds in starch (Van Zyl et al., 2012). At 65 °C, glucoamylase is added to further break down α-1,4 and α-1,6 glycosidic bonds to release glucose monomers that are converted into ethanol by *S. cerevisiae*. After solids removal (Li et al., 2022), the ethanol is concentrated via distillation and dehydration. Wet milling is similar to dry milling, except that the feedstock is steeped and debris is separated before enzymatic hydrolysis and fermentation. Although wet milling involves higher costs, it yields value-added by-products such as sweeteners, syrups, and oils (Jain & Kumar, 2024).

Second-generation bioethanol (advanced biofuels) relies on complex biomass rich in starch, lipids, proteins, and lignocellulose, such as bagasse from sugarcane and sweet sorghum, rice husks, corn stover, straw, grass, and paper sludge (Jain & Kumar, 2024). Cellulose and hemicellulose are hydrolyzed using either acids like HCl or H_2SO_4, enzymes such as endo-(1,4)-β-D-glucanase, exo-(1,4)-β-D-glucanase, and β-glycosidases, hot water (known as autohydrolysis), or steam explosion to release fermentable sugars (Joyia et al., 2024; Shukla et al., 2023). Lignocellulosic biomass, consisting of cellulose, hemicelluloses, and lignin, is naturally resistant to enzymatic conversion and requires advanced, and more costly, hydrolytic processes.

Third-generation bioethanol produced from macro- and microalgae is considered more sustainable because algae can be cultivated in wastewater and require less land (Rocher et al., 2025). The high carbohydrate content (35%–74% on a dry weight basis), the lack of lignin, the ability to absorb CO_2, and the overall low carbon footprint of algae are appealing features (Devarapalli & Atiyeh, 2015). For example, *Ecklonia maxima* grown with a recombinant strain of *S. cerevisiae* expressing mannitol dehydrogenase, alginate lyase, and laminarinase produced 10.30 g/L ethanol (Rocher et al., 2025).

The feedstock used for bioethanol production is not sterilized, allowing naturally occurring fungi and bacteria to compete with *S. cerevisiae* for fermentable carbohydrates and nutrients, often leading to reduced ethanol yields and slow or halted fermentation (Azhar et al., 2017; Figure 2). Because sterilization is neither economically feasible nor practical on an industrial scale, various strategies are used to control microbial contamination. These include pretreating the biomass with an "acid-wash" or adding chlorine dioxide (ClO_2), sulphur dioxide (SO_2), or hydrogen peroxide (H_2O_2) (Seo et al., 2020). Although antibiotics such as penicillin, erythromycin, virginiamycin, streptomycin, and tetracycline have been added to biofuel feedstocks, this practice is strongly discouraged because it can select for antibiotic-resistant microorganisms. Natural products with antibacterial properties, such as hops and propolis, have shown potential when added to the fermenting biomass at dosages of 10–100 mg/L (Ceccato-Antonini, 2018). Microbial contamination may also be controlled by adding antimicrobial peptides (AMPs), which also have applications in agriculture, food, human, and veterinary sciences (Huan et al., 2020).

Bacteriocins, naturally produced by lactic acid bacteria (LAB), were initially thought to be effective only against bacteria, but several broad-spectrum bacteriocins and bacteriocin-like peptides have been reported (Simons et al., 2020; Sugrue et al., 2024). The US Food and Drug Administration (FDA) has approved only a few AMPs to date, and some have been evaluated in clinical trials (Chén & Lu, 2020). However, the large-scale production and purification of bacteriocins from bacteria are costly and labour-intensive (Vermeulen et al., 2020). An alternative is to express recombinant AMP genes in yeast strains. Several groups (reviewed by Cao et al., 2018) have reported the expression of bacteriocins in *Pichia pastoris* (renamed as *Komagataella phaffii*), but only a few have documented their production in *S. cerevisiae* (Rossouw et al., 2024, 2025; Schoeman et al., 1999; Van Reenen et al., 2003).

This review provides a concise overview of microbial contamination in commercial starch-to-ethanol fermentations, which are particularly vulnerable to LAB, and methods to control these contaminants. It examines whether yeast strains (particularly *S. cerevisiae*) can produce recombinant AMPs to control bacterial contamination, and explores molecular strategies to enhance the efficacy of these strains in industrial processes. Finally, it highlights the potential metabolic burden of recombinant proteins on host cells and acknowledges regulatory challenges for the use of genetically modified organisms in industrial fermentations.

Microbial control in starch-based fermentations

Plant material is naturally contaminated by microorganisms during harvesting, transportation, storage, and processing (Brexó & Sant'Ana, 2017; Rich et al., 2018). These contaminants include LAB (Bonatelli et al., 2017), which are well adapted to survive in acidic conditions (<pH 3), high ethanol levels (8% v/v), and low-oxygen environments typically associated with bioethanol fermentations (Papadimitriou et al., 2016). For example, *Lactobacillus* spp. inhibits the growth of *S. cerevisiae* by competing for nutrients and fermentable carbohydrates, lowering the pH, and producing diacetyl, reuterin, and fatty acids (Brexó & Sant'Ana, 2017). Various strategies have been employed to control the growth of LAB and other bacterial contaminants, as discussed in the following sections.

Chemical treatment

Chemical agents, such as H_2O_2, ClO_2, SO_2, ammonia (NH_3), acrinol, and acids (including sulphuric, phosphoric, tartaric, and aspergillic acid) are often used to control microbial contamination of feedstock. Acid-washing of yeast cells is frequently employed in bioethanol production plants to inhibit the growth of *Lactobacillus* spp. (Basso et al., 2008). However, this process reduces yeast cell viability, potentially leading to sluggish or stuck bioethanol production. Treatment with

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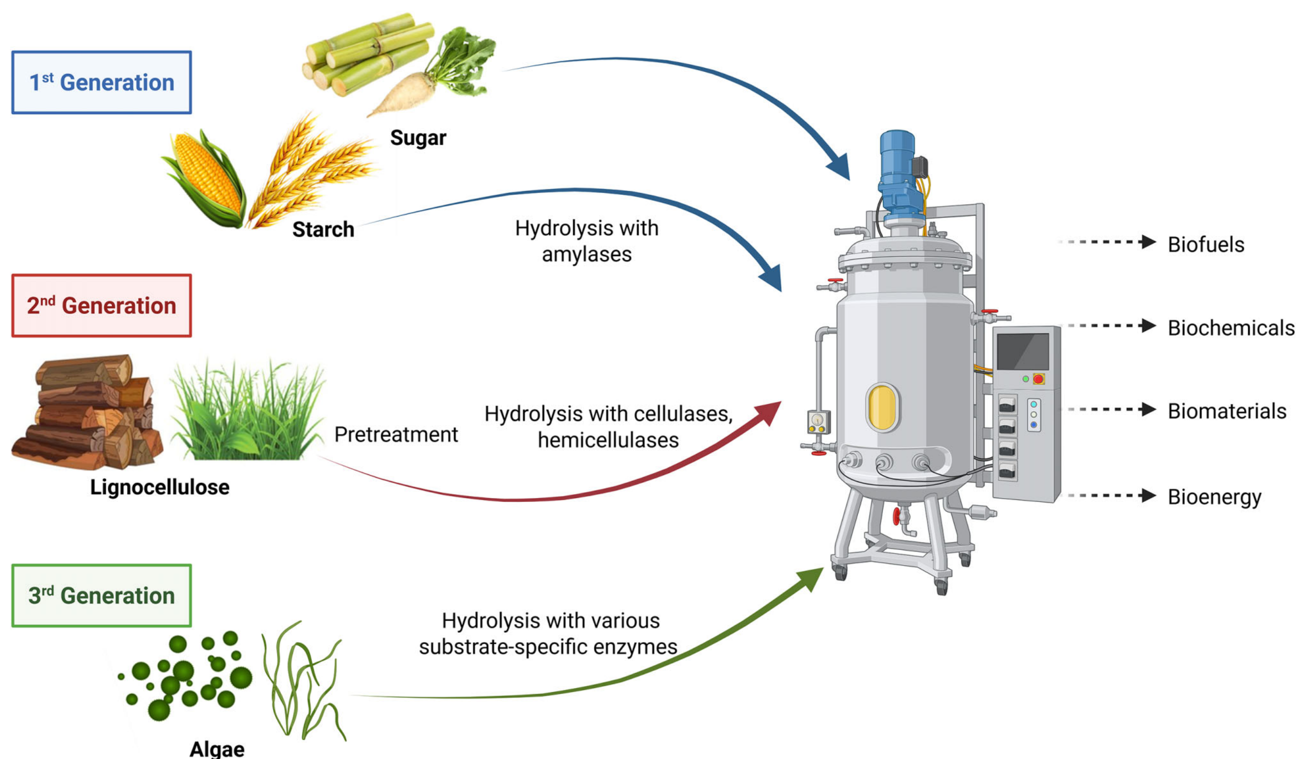

Fig. 1 Overview of biofuel generations based on different substrates and hydrolysis processes, and potential products that may be derived (created in https://BioRender.com).

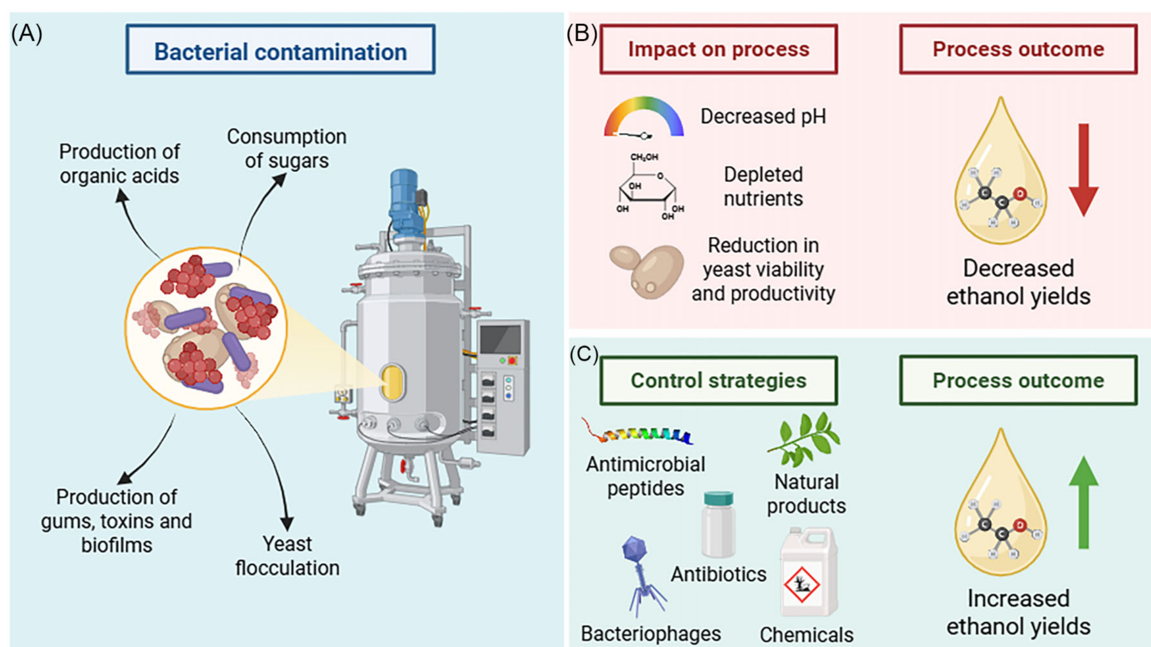

Fig. 2 Bacterial contamination has a major impact on bioethanol fermentations (A), which leads to multiple negative process outcomes (B). Various control strategies (C) can be employed to reduce contamination and achieve a favourable process outcome (adapted from Ceccato–Antonini, 2018; Sekoai et al., 2019; Seo et al., 2020; created in https://BioRender.com).

0.5%–1.5% (w/v) ammonia notably decreased LAB and mould growth during corn grain fermentation, resulting in a 2% increase in ethanol production (Broda & Grajek, 2009). Urea hydrogen peroxide (UHP) at 30–32 mmol/L significantly suppressed the growth of *Lactiplantibacillus plantarum*, *Lacticaseibacillus paracasei*, *Lacticaseibacillus rhamnosus*, *Limosilactobacillus fermentum*, and *Lactobacillus* sp. strain 3 in fermented wheat mash, without inhibiting the growth of *S. cerevisiae* (Narendranath et al., 2000). Chemical treatment can effectively reduce

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bacterial contamination, but high concentrations may damage yeast cells or pose environmental hazards (Seo et al., 2020).

Antibiotics

Antibiotics are widely used to control bacterial contamination, including penicillin, tetracycline, virginiamycin, streptomycin, erythromycin, and monensin, or combinations of these (Rich et al., 2018). In the biofuels industry, antibiotics are typically used at concentrations of 0.1–20 mg/L (Seo et al., 2020), compared to generally higher dosages used in the veterinary industry, i.e., 8–10 mg penicillin/kg body weight for cats and dogs, 24 mg/kg for cattle, and 10–110 mg virginiamycin/kg for swine and cattle (Mercer, 2022; Vidal et al., 2022). The use of antibiotics in the biofuels industry requires significant quantities, making this approach very expensive. Concerns have been raised about the global increase in antibiotic resistance, driven by the widespread use and continuous exposure of microorganisms to antibiotics.

Numerous studies have documented the presence of antibiotic-resistant bacteria in bioethanol production facilities (Ceccato–Antonini, 2018; Mendonça et al., 2016). Since antibiotic residues remain in the fermentation broth or co-products, disposal of industrial waste into the environment could encourage the development of resistant strains. Bischoff et al. (2016) detected biologically active virginiamycin in grain by-products from corn-based bioethanol fermentations. The presence of antibiotic residues in dried distillers grains with solubles (DDGS) may exceed regulatory limits and significantly affect the economic feasibility of ethanol plants and animal health (Sankarlal et al., 2025). This calls for the development of alternative methods to control microbial contamination in bioethanol production.

Natural compounds

Antimicrobial compounds from natural sources have been used for centuries to treat human infections and prevent food and beverage spoilage. Hop acid, extracted from hops, has reduced bacterial contamination in bioethanol fermentations by at least twofold (Madaleno et al. 2016). Chitosan, a chitin-derived polysaccharide mainly found in the shells of shrimp, crabs, and lobsters, exhibits broad-spectrum antimicrobial activity against bacteria, yeast, fungi, and algae (Atay, 2020). However, the mode of antimicrobial action varies for different microorganisms, and studies have so far been conducted only at the laboratory scale (Ke et al., 2021).

Bacteriophages

Bacteriophages are crucial in aquatic environments and are also present in soil, food, and the digestive systems of humans and animals (Dion et al., 2020). Because they target specific species and strains, they minimize the risk of harming nontarget or beneficial bacteria. Bacteriophages produce endolysins that degrade peptidoglycan in bacterial cell walls, leading to cell lysis. However, the potential of tendolysins to control bacterial contamination in bioethanol fermentations remains poorly documented (Liu et al., 2015; Roach et al., 2013). The synthetic *LysMP* gene derived from the prophage sequence of *L. fermentum* KGL7 was expressed in *Escherichia coli*, resulting in a > 4-log reduction in the number of live cells. This reduction correlated with decreased levels of lactic and acetic acids and increased ethanol production during yeast corn mash fermentations (Patel et al., 2023). Furthermore, expressing the endolysin LysKB317 in *S. cerevisiae* increased ethanol output by 16% (Lu et al., 2024).

The widespread application of bacteriophage endolysins in the bioethanol industry would require high concentrations of the purified lytic enzymes, making this treatment method very expensive. Bacteriophage resistance could be addressed by combining bacteriophages with a secondary treatment method (e.g., antibiotics, chemicals, or physical treatments) or by using bacteriophage cocktails (mixtures of different bacteriophages) (Bertozzi Silva & Sauvageau, 2014; Herridge et al., 2020).

Antimicrobial peptides

Various research groups have investigated AMPs as alternative antimicrobial agents (Huan et al., 2020; Lei et al., 2019; Magana et al., 2020). These small, cationic peptides (10–100 amino acids) carry a net positive charge and have an amphipathic structure (Meng et al., 2019). AMPs have been identified in various lifeforms as part of the innate immune system as the first line of defence against pathogens (Mahlapuu et al., 2016). In addition to their broad-spectrum activity against Gram-positive and Gram-negative bacteria, fungi, and viruses (Gani et al., 2025), AMPs are nontoxic and can modulate the immune response (Duarte-Mata & Salinas-Carmona, 2023), positioning them as promising candidates for the development of new therapies.

AMPs are classified based on their source, activity, structure, and amino acid composition (Huan et al., 2020). They primarily work by physically disrupting the microbial cell membrane or crossing it to attack internal components (Mahlapuu et al., 2016). Cationic AMPs interact electrostatically with negatively charged bacterial membranes and insert themselves into the membrane. Targeting the membrane without specific receptor engagement reduces the risk of resistance development (Tan et al., 2021). The permeability of the cell membrane allows ions and metabolites to escape, causing cell rupture and lysis (Ma et al., 2024). Membrane permeabilization enables some AMPs to translocate into the cytoplasm, where they disrupt essential processes such as DNA and RNA synthesis, translation, protein folding, enzymatic activity, and cell wall synthesis (Yeaman & Yount, 2003). Four main mechanisms have been proposed for membrane permeabilization.

The "barrel stave" model explains how peptides form transmembrane pores by embedding into the membrane bilayer. The "toroidal pore" hypothesis proposes that peptide insertion causes phospholipids to bend smoothly, forming a channel lined with both peptides and phospholipid heads. According to the "carpet" model, peptides contact the membrane surface, induce tension, and cause micelle formation, leading to membrane disruption (Mahlapuu et al., 2016). The "aggregate" concept describes AMPs binding to the cytoplasmic membrane to form a peptide-lipid complex, allowing entry into the cytoplasm to target intracellular components (Zhang et al., 2021).

A closer look at bacteriocins

Bacteriocins are small (30–60 amino acids), positively charged, amphipathic AMPs produced by both Gram-positive and Gram-negative bacteria, with differences in size, mode of action, and activity (Pircalabioru et al., 2021). Usually, "bacteriocin" refers to peptides from Gram-positive bacteria, while those from Gram-negative bacteria are called "colicins" or "microcins." Typically, Gram-positive bacteriocins are smaller than 10 kDa and are effective at low concentrations,

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whereas Gram-negative bacteriocins are usually larger than 20 kDa. Bacteriocins tend to be less effective against Gram-negative bacteria because of the outer membrane barrier.

Types of bacteriocins

Bacteriocins produced by Gram-positive bacteria are divided into four classes, each with distinct mechanisms of action against target organisms (Figure 3). Class I bacteriocins (<5 kDa), consisting of 19–50 amino acids, are known as lantibiotics because they contain lanthionine residues (Pircalabioru et al., 2021). These bacteriocins undergo several post-translational modifications during biosynthesis and contain uncommon amino acids. Examples of this class include lanthipeptides, sactibiotics, and labyrinthopeptins (Komori et al., 2025). Class II bacteriocins, also known as nonlantibiotics, constitute the majority of naturally occurring bacteriocins (Drider et al., 2006) and are mainly produced by food-associated LAB, such as *Lactobacillus, Enterococcus, Pediococcus*, and *Leuconostoc* (Zhang et al., 2022a). Unlike class I bacteriocins, they do not undergo extensive post-translational modifications, aside from disulphide bond formation. These peptides are classified into four subclasses: class IIa or "pediocin-like" bacteriocins (named after pediocin PA-1); class IIb, or two-peptide bacteriocins, which need two different peptides to be produced simultaneously for activity; class IIc, including circular bacteriocins; and class IId, which comprises linear non-pediocin-like single-peptide bacteriocins, leaderless peptides, and multipeptide bacteriocins (Cui et al., 2012; Ennahar et al., 2000). Class III bacteriocins are large (>10 kDa), heat-sensitive peptides. Class IIIa comprises bacteriolysins, proteins that break down bacterial cell walls, such as enterolisin A (Pircalabioru et al., 2021). Class IIIb comprises nonlytic proteins that eliminate target cells by disrupting the plasma membrane potential, without causing cell lysis (Darbandi et al., 2022). Class IV peptides contain lipid or carbohydrate parts, making them vulnerable to glycolytic and lipolytic enzymes (Simons et al., 2020).

Peptides produced by Gram-negative bacteria are mainly divided into four groups (Figure 3): *E. coli* produces large peptides called colicins, ranging from 30 to 80 kDa (Zimina et al., 2020). Colicin-like bacteriocins are similar in size but are produced by bacteria other than *E. coli*. Enterobacteriaceae produce microcins, which are small peptides (<10 kDa). The largest peptides are tailocins (20–100 kDa), which have a narrow activity range and resemble bacteriophage tails (Fernandez et al., 2020).

Structure and production of native Class IIa bacteriocins

LAB primarily produce Class IIa bacteriocins that target related LAB and various bacteria responsible for food spoilage and disease, including *Listeria monocytogenes, Staphylococcus aureus*, *Clostridium perfringens, Bacillus cereus,* and *Enterococcus faecium* (Simons et al., 2020). Sequence alignment (Figure 4A and B) reveals that class IIa bacteriocins share a conserved N-terminal YGNGV motif (the pediocin box), with a varying hydrophobic or amphiphilic C-terminus (Cui et al., 2012). They usually contain two or four cysteine residues that form one or two disulphide bonds, which are required for structure and activity. The N-terminal domain has a β-sheet-like structure stabilised by a conserved disulphide bond, while the C-terminal domain contains one or two α-helices, often ending with an extended tail. Some bacteriocins, such as pediocin PA-1, enterocin A and plantaricin 423, also possess an additional disulphide bond at the C-terminal to maintain their three-dimensional stability (Figure 4C). Several studies have shown that this additional disulphide bond enhances antimicrobial effectiveness (Eijsink et al., 1998; Fimland et al., 2000).

Class IIa bacteriocins disrupt bacterial cell membranes by forming ion-selective pores, which collapse the proton motive force and reduce intracellular ATP levels (Christensen & Hutkins, 1992). Liang et al. (2025) reported that pediocin-like bacteriocins specifically bind to receptors such as the Man-PTS on bacterial membranes, inserting into the membranes to form pores (Figure 4D). The pediocin box likely serves as a recognition sequence for the membrane-bound protein receptor [protein IIC (MptC)] of Man-PTS in target cells. In bacteria, sugars are imported and phosphorylated through the phosphotransferase system (Drider et al., 2006). By inhibiting sugar transport—essential for bacterial growth—the bacteriocin's interaction with the IIC docking protein disrupts the Man-PTS system, leading to membrane permeabilization and cell death (Wu et al., 2020).

Various genes within class IIa bacteriocin operons control bacteriocin production by native producers, resulting in fluctuating or limited peptide output. The operon typically contains genes for the prepeptide, immunity proteins, an ATP-binding cassette (ABC) transporter, accessory proteins for extracellular translocation, proteins involved in disulphide bond modifications, and regulatory elements (Figure 5; Nes et al., 1996). It also includes auxiliary protein genes that are crucial for bacteriocin synthesis, facilitating leader peptide processing and the membrane translocation of the mature peptide (Zhang et al., 2022b). Some class IIa bacteriocins, such as pediocin PA-1, require accessory proteins, such as PedC, for proper disulphide bond formation, which is vital for their activity (Oppegård et al., 2015).

Class IIa bacteriocins are first produced as prebacteriocins, which include an N-terminal leader sequence that renders them inactive and protects the producer (Håvarstein et al., 1995). This leader peptide likely functions as a recognition signal for the suitable ABC transporter (Ennahar et al., 2000). Usually, the leader consists of 15–30 amino acids and features a double-glycine motif that serves as a cleavage site at the N-terminus of the transmembrane domain, facilitating the release of the mature peptide (Zhang et al., 2022b). When the prebacteriocin binds to the N-terminal domain, it activates ATP hydrolysis and induces conformational changes in the transporter, resulting in cleavage of the leader peptide and export of the mature peptide. However, some class IIa bacteriocins lack the double-glycine motif and instead have a hydrophobic, Sec-dependent N-terminal leader that is removed during translocation by signal peptidase.

Bacteriocin-producing bacteria protect themselves from their own bacteriocins by coexpressing immunity proteins (Fimland et al., 2002). The immunity proteins (81–115 amino acids) show substantial sequence variability that affects their specificity (Zhang et al., 2022b). The C-terminal domain of these proteins is crucial for the specific recognition of their corresponding bacteriocins. Two models have been proposed to explain their function: either they directly interact with the bacteriocin to prevent pore formation or bind to the cytoplasmic side of the receptor to inhibit its interaction with the bacteriocin (Figure 5). Bacteriocins are categorized into subgroups based on the type of immunity protein (Vermeulen et al., 2021). For example, plantaricin 423 and enterocin A contain type 2 immunity proteins, whereas mundticin ST4SA contains a type 1 immunity protein, resulting in different susceptibility profiles among target organisms.

Class IIa bacteriocin synthesis is controlled by quorum sensing involving key proteins such as an inducing peptide, a membrane-bound

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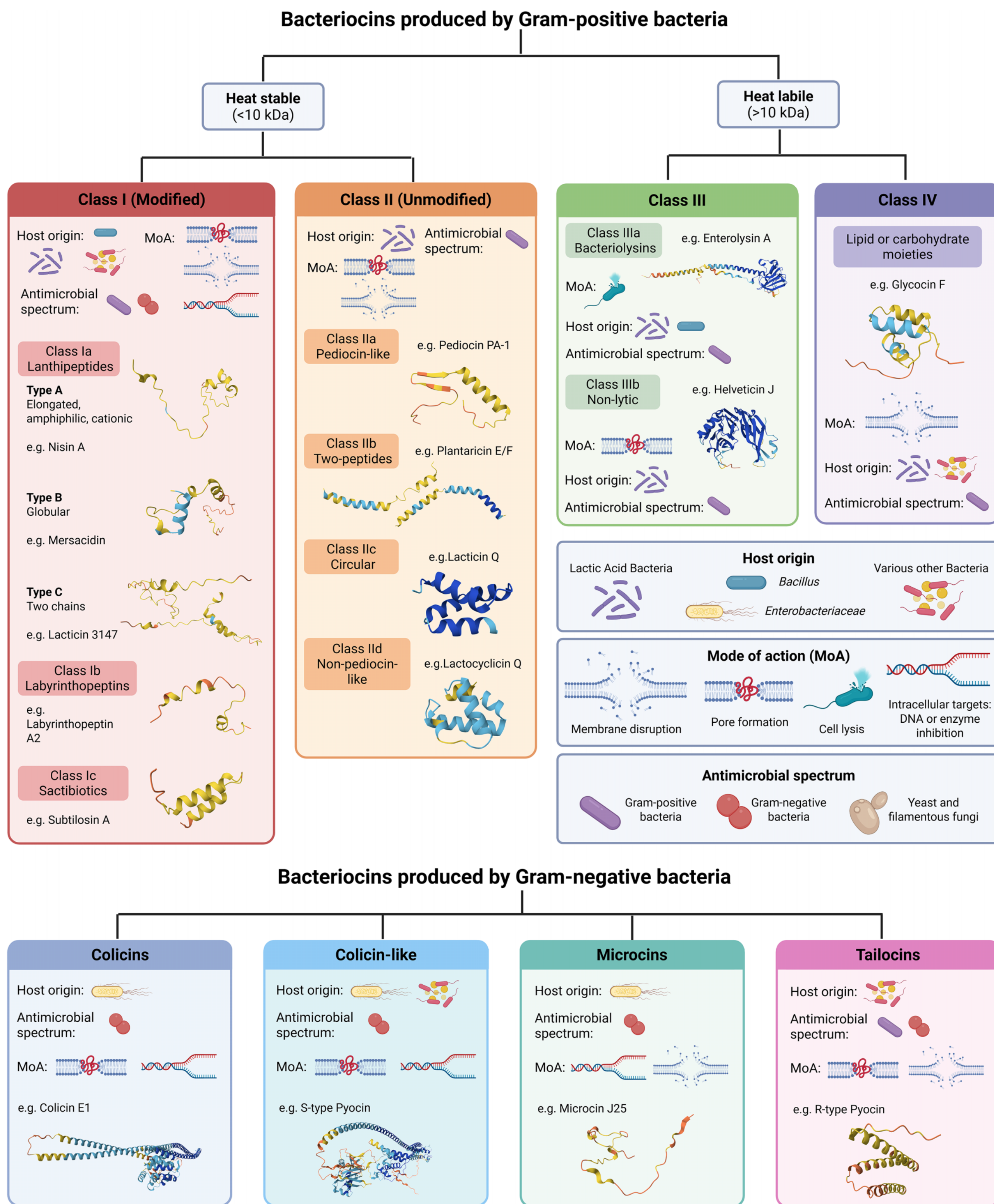

Fig. 3 Summary of the different classes of bacteriocins produced by Gram-positive and Gram-negative bacteria, including their antimicrobial spectra, mechanisms of action, origins and peptide structures. Created in https://BioRender.com; peptide structures prepared with AlphaFold (Jumper et al., 2021).

histidine kinase, and a cytoplasmic response regulator. These components collaborate to activate the bacteriocin biosynthetic gene cluster (Zhang et al., 2022b). The inducing peptide, initially produced as a prepeptide with an N-terminal leader sequence, is cleaved during secretion by the ABC transporter. As bacterial populations increase, the inducer peptide's concentration rises, prompting autophosphorylation of the histidine kinase and phosphate transfer to the response regulator. This sequence ultimately induces the expression of the bac-

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(A)

Leucocin A	K Y Y G N G V H C T K S G C S V N W G E A F S A G V H R L A N G G N G F W
Leucocin B	K Y Y G N G V H C T K S G C S V N W G E A F S A G V H R L A N G G N G F W
Sakacin P	K Y Y G N G V H C G K H S C T V D W G T A I G N I G N N A A A N W A T G G N A G W N K
Enterocin HF	K Y Y G N G V S C N K K G C S V D W G K A I G I I G N N A A A N L T T G G K A G W K G
Mesentericin Y105	K Y Y G N G V H C T K S G C S V N W G E A A S A G I H R L A N G G N G F W
Mundticin ST4SA	K Y Y G N G V S C N K K G C S V D W G K A I G I I G N N S A A N L A T G G A A G W K S

(B)

Divercin V41	K Y Y G N G V Y C N S K K C W V D W G Q A S G C I G Q T V V G G W L G G A I P G K C
Enterocin A	T T H S G K Y Y G N G V Y C T K N K C T V D W A K A T T C I A G M S I G G F L G G A I P G K C
Coagulin	K Y Y G N G V T C G K H S C S V D W G K A T T C I I N N G A M A W A T G G H Q G T H K C
Sakacin G	K Y Y G N G V S C N S H G C S V N W G Q A W T C G V N H L A N G G H G V C
Pediocin PA-1	K Y Y G N G V T C G K H S C S V D W G K A T T C I I N N G A M A W A T G G H Q G N H K C
Plantaricin 423	K Y Y G N G V T C G K H S C S V N W G Q A F S C S V S H L A N F G H G K C

(C) (D)

Fig. 4 Sequence alignment and grouping of class IIa bacteriocins with (A) one or (B) two disulphide bonds. The cysteine residues involved in the disulphide bonds are highlighted in grey. (C) Pediocin PA-1 primary structure, indicating the disulphide bonds (S–S), cysteine residues in yellow, and the pediocin box in blue residues (prepared using AlphaFold; Jumper et al., 2021). (D) Recognition of the pediocin box by the mannose phosphotransferase system (Man-PTS) on the cell membrane of the target organism. Created in https://BioRender.com.

teriocin biosynthetic gene cluster (Figure 5). Additionally, factors such as temperature and divalent metal cation levels can affect bacteriocin production by influencing the binding of the inducer peptide to the histidine kinase (Vermeulen et al., 2021).

Environmental factors, including media composition, pH, temperature, salt levels, and microbial growth kinetics, influence bacteriocin production (Zhang et al., 2022b). Research on fermentation indicates that bacteriocin synthesis aligns with primary metabolite patterns, occurring during exponential growth and decreasing as cells reach the stationary phase. Additionally, producing bacteriocins with LAB requires complex and costly growth media, increasing the cost of large-scale production. Media such as CM, MRS, SM8, and M17 are used to culture bacteriocin-producing LAB, but their complex nutrient profiles make peptide purification more challenging.

Common approaches to purifying class IIa bacteriocins from culture supernatants include protein precipitation at various salt concentrations, followed by separation methods like gel filtration, hydrophobic-interaction chromatography, ion-exchange chromatography, or reverse-phase high-performance liquid chromatography (HPLC) (Drider et al., 2006). Nonetheless, obtaining pure class IIa bacteriocins remains challenging due to low yields, which often make purified peptides less suitable for commercial application. Usually, a partially purified peptide, a crude bacterial fermentate, or bacteriocin-producing bacteria are used directly.

Only a few bacteriocins are currently used as food preservatives, primarily nisin A, pediocin PA-1, and nisin Z (Sugrue et al., 2024). Since bacteriocins are small, they can be chemically synthesised. However, chemical synthesis of AMPs is expensive, making large-scale commercial use difficult (Sadeeq et al., 2025). Extracting AMPs from other natural sources, such as higher eukaryotic hosts, can be challenging and time-consuming. Heterologous expression of AMPs could enable more cost-effective and straightforward production of these valuable compounds.

Commercial applications

Class IIa bacteriocins serve as food preservatives and agents against disease (Lahiri et al., 2022). They target related LAB as well as various food spoilage and pathogenic bacteria, including *L. monocytogenes*, *S. aureus*, *C. perfringens*, *B. cereus*, and *E. faecium* (Simons et al., 2020). While these bacteriocins show activity against several important pathogens, they are particularly effective against the foodborne pathogen *L. monocytogenes* (Darbandi et al., 2022).

As indicated in Figure 6, bacteriocins can be used as food preservatives, either added directly or produced during fermentation by starter cultures (Liang et al., 2025). Currently, only a few, such as nisin A, enterocin AS-48, and enterocin 416K1, have FDA approval for use as food additives (reviewed by Nisar et al., 2024). In the food industry, they are utilized as partially purified compounds, crude fermentates, or protective cultures that produce bacteriocins (Chikindas et al., 2018). Research focuses on enhancing the effectiveness of bacteriocins in food settings by developing derivatives with greater resistance to proteolytic enzymes, improved activity, and broader inhibitory ranges. Moreover, bacteriocin-producing organisms could be integrated into functional foods or over-the-counter products to modulate the gastrointestinal (GI) microbiota.

The probiotic and antimicrobial properties of bacteriocins have positioned them as promising candidates for applications in ani-

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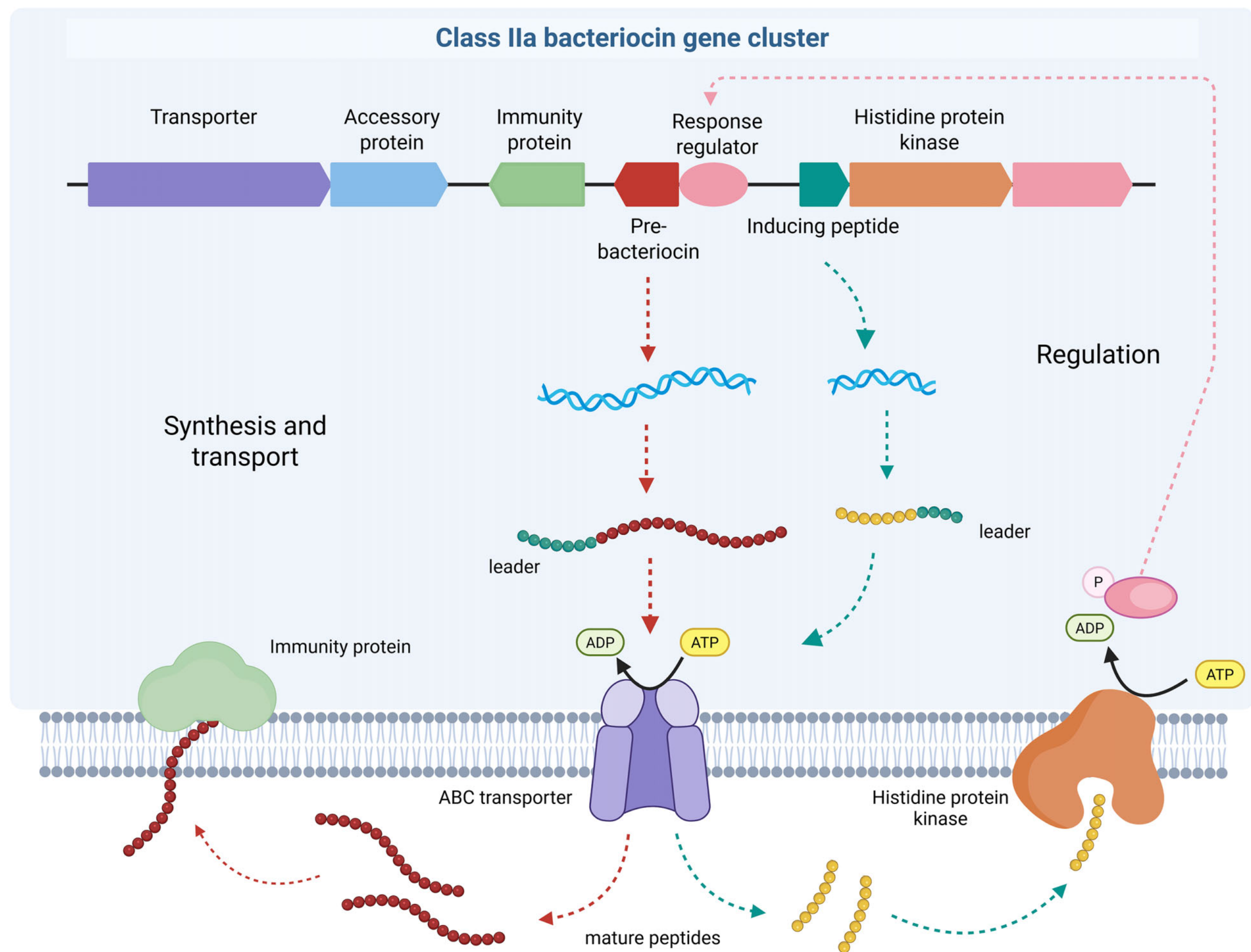

Fig. 5 A simplified overview of the different components involved in class IIa bacteriocin synthesis and regulatory processes (created in https://BioRender.com).

mal farming, including livestock, poultry, and aquaculture production systems (reviewed by Meskhi et al., 2026). Bacteriocins have shown great promise in modulating the gastrointestinal microbiota of broiler chickens and controlling the proliferation of opportunistic pathogens, such as *Clostridium perfringens*. Commercial products containing nisin as the active ingredient have been reported as effective against pathogenic *Staphylococcus aureus* and *Streptococcus* species (including *Streptococcus uberis* and *Streptococcus agalactiae*), the main pathogens causing mastitis in dairy cows. There is also significant interest in the application of bacteriocin-producing probiotic strains as feed supplements in aquaculture to promote growth and reduce infections.

Recent technological advances have enabled bacteriocins to evolve from food applications to human health, including their use in treating infections (Chikindas et al., 2018; Huan et al., 2020; Zhang et al., 2021). Additionally, several properties, particularly peptide stability and toxicity, can be enhanced when AMPs are incorporated into specialized delivery systems, with polymeric nanoparticles recently gaining popularity (Thakur et al., 2022). Chikindas et al. (2018) suggested that bacteriocins primarily serve as signals and repellents to bacteria, rather than as agents that kill them. However, at concentrations higher than those naturally present, some bacteriocins exhibit additional roles, such as disrupting membranes, forming pores, interfering with cell division, or inducing cell lysis. These effects depend on the specific bacteriocin and target microorganism and can occur at different concentrations.

Given the diverse strategies employed by bacteriocins, combining multiple types could broaden the target range of antimicrobial action against bacteria. Additionally, the antibacterial effect of the bacteriocin-producing yeast against LAB in bioethanol fermentations could be enhanced by using it alongside other biological control strategies. For instance, several studies have shown that combination therapies involving bacteriophages or AMPs with antibiotics exhibit synergistic antimicrobial activity and can even resensitise bacteria to antibiotics previously considered resistant (Osman et al., 2023; Taheri-Araghi, 2024).

Limited research exists on the use of class IIa bacteriocins to combat bacterial contamination in the biofuels industry, although some have shown promise. For instance, pediocin PA-1 can inhibit bacterial growth during alcoholic fermentations due to its stability over a wide temperature range (−80 to 100 °C) and pH range (pH 2–10) (Khorshidian et al., 2021). Limayem et al. (2011) reported that nisin controlled the growth of various contaminating *Lactobacillus* spp. in bioethanol fermentations, with 0.05 mg/ml nisin reducing strain growth by 6 log CFU/ml after 48 h. However, deploying bacteriocins in the bioethanol industry requires scaling up production and purification, which pose challenges such as high costs and low yields from native hosts (Parachin et al., 2012). Additional hurdles

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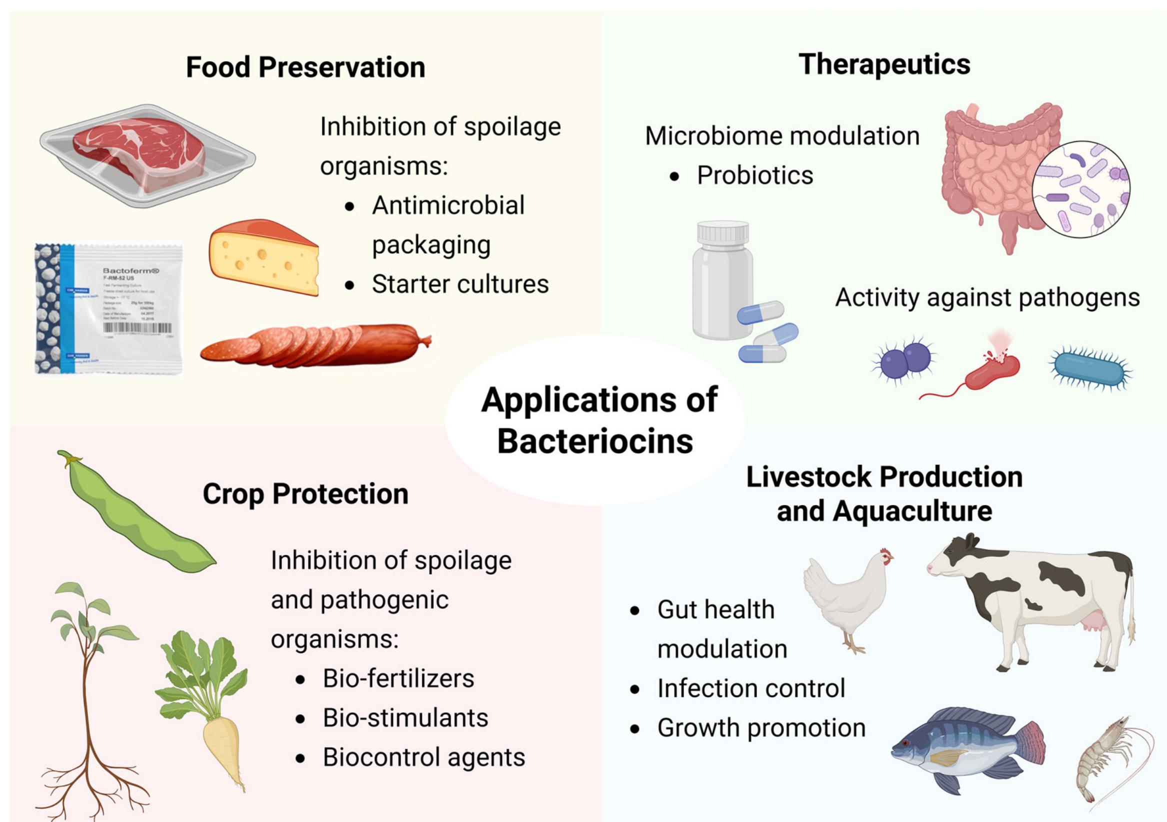

Fig. 6 Commercial applications of bacteriocins in food preservation, therapeutic applications, agriculture, aquaculture, animal husbandry and poultry farming (created in https://BioRender.com).

include sensitivity to proteolytic enzymes and a limited target spectrum, mainly affecting Gram-positive bacteria with limited activity against Gram-negative bacteria (Parada Fabián et al., 2025). Liang et al. (2025) emphasized that maximizing bacteriocin yields depends on careful control of initial fermentation pH and dissolved oxygen levels.

Genetic engineering techniques could reduce the high cost of producing pure bacteriocins by recombinant expression of these peptides. The most common host for recombinant protein production is *E. coli*, but peptide production is hampered by the bacterial host's sensitivity towards AMPs. Production of AMPs, especially bacteriocins, in yeast offers a promising solution as bacteriocins do not target yeast and show no risk of toxicity (Rossouw et al., 2024). Having established the background and applications of bacteriocins, this review examines previous studies on recombinant AMP production using different microbial expression systems to evaluate and highlight the potential of genetically modified yeast strains for bacteriocin production.

Heterologous expression of AMPs

Recombinant gene technology involves inserting foreign genes into specific vectors or linear cassettes for expression in prokaryotic or eukaryotic host cells (Parachin et al., 2012). This process facilitates large-scale production of recombinant proteins for a range of industrial uses. Recent innovations, such as artificial intelligence and machine learning, could accelerate AMP discovery and optimization. Some models predict activity by examining chemical structures and molecular features or by screening databases and virtual libraries for potential candidates (Sadeeq et al., 2025). Different recombinant hosts, including bacteria, yeast, plants, and insect cells, have been studied, each with specific pros and cons. The primary hosts for recombinant protein production are bacteria and yeast, but the choice of an AMP host depends on target specificity, post-translational modifications, and protein secretion, among others. Although bacteriocins are the principal AMPs considered in this review, studies on their heterologous production in microbial hosts remain limited. The following sections highlight some of the production systems and considerations for AMP expression in general, with specific reference to bacteriocins where relevant.

Bacterial expression systems

Escherichia coli often serves as a host for foreign protein production, but AMPs can be toxic to bacterial cells (Rosano & Ceccarelli, 2014). Another limitation of *E. coli* is its lack of natural protein secretion, which leads to the accumulation of nonfunctional proteins, insoluble inclusion bodies, and increased protein degradation by intracellular proteases (Li, 2011). These problems can be alleviated by using signal peptides to promote peptide secretion and by using fusion proteins to reduce AMP toxicity. Additionally, bacterial expression systems typically cannot perform certain post-translational modifications, which limits the heterologous expression of modified bacteriocins like lantibiotics and circular bacteriocins. Consequently, coexpressing accessory proteins is required to facilitate these modifications.

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Various class IIa bacteriocins, such as pediocin PA-1 (Liu et al., 2011; Nguyen et al., 2020), divercin V41 (Ingham et al., 2005), plantaricin Pln1 (Meng et al., 2016), plantaricin E (Meng et al., 2017a), enterocin P (Le et al., 2014), mundticin ST4SA and plantaricin 423 (Vermeulen et al., 2020), have been successfully expressed in *E. coli*. Vermeulen et al. (2020) fused the His-tagged green fluorescent protein (GFP) to mature plantaricin 423 and mundticin ST4SA in *E. coli*, thereby reducing toxicity during expression and enabling peptide monitoring via GFP autofluorescence. Still, purification via immobilized metal affinity chromatography and cleavage were required to free the peptides and restore activity, increasing production costs and resulting in low yields and conformational isomers of plantaricin 423. Alternatively, Weixler et al. (2022) produced recombinant nisin using *Corynebacterium glutamicum* in a two-step process, i.e., biosynthesis and export of fully modified prenisin, yielding 1.25 mg/L of active nisin.

Yeast expression systems

Yeast strains offer a promising alternative for bacteriocin production, since class IIa bacteriocins do not affect yeast cells. Yeasts can also perform post-translational modifications, including disulphide bond formation facilitated by the endoplasmic reticulum (ER) oxidoreductin and protein disulphide isomerase (Sevier & Kaiser, 2002). They are effective at secreting peptides into the extracellular medium, thereby boosting protein yields with few purification steps (Ahmad et al., 2014). Most research has centred on *P. pastoris* [now called *K. phaffii*] as an AMP host, with only a few studies successfully expressing AMPs in *S. cerevisiae* (Tables 1 and 2).

Pichia pastoris (*K. phaffii*) favors producing recombinant proteins over ethanol, and can be grown to high cell densities, which is beneficial for industrial-scale AMP production. Secretion of recombinant AMPs by *P. pastoris* simplifies downstream purification, which is essential for large-scale manufacturing (Meng et al., 2019). Most research on bacteriocin expression uses the methanol-inducible *P. pastoris AOX* promoter, but methanol adds extra costs and complicates purification and downstream processes (Table 1). This host has produced recombinant peptides at levels ranging from 10 µg/L to more than 700 mg/L (Burrowes et al., 2005; Cao et al., 2018). Different peptide classes have been successfully expressed in *P. pastoris*, including the class IIa bacteriocin enterocin A (Hu et al., 2014), insect AMPs such as apidaecin and abaecin from honeybees (Cao et al., 2018; Chen et al., 2017; Luiz et al., 2017), and cecropins from silk moths (Guo et al., 2012; Wang et al., 2011). Zhao et al. (2024) produced a dimeric AMP, LIG, which was created by fusing the human-derived peptide LL-37 with bovine neutrophil-secreted indolicidin.

Saccharomyces cerevisiae has been used extensively for the production of various commercially significant proteins and also has potential for large-scale production of bacteriocins (Kim et al., 2015). As shown in Table 2, only a few studies have reported the expression of AMPs in *S. cerevisiae*, including two from higher eukaryotes. Xia et al. (2013) expressed cecropin XJ from *Bombyx mori* in *S. cerevisiae*, with the recombinant peptides exhibiting activity against Gram-positive and Gram-negative bacteria. The expression was driven by the *GAL1* promoter, and a His-tag was used for purification. More recently, Jiang et al. (2021) cloned and expressed the *Ascaris suum* cecropin P1 in *S. cerevisiae* through induced expression, yielding 7.83 mg/L of purified peptide that effectively inhibited *E. coli, Shigella*, *Salmonella* and *Pasteurella* species.

Only a few LAB bacteriocins have been expressed successfully in *S. cerevisiae*, with varying degrees of success (Table 2). Schoeman et al. (1999) reported the production of native pediocin PA-1, with 16-fold higher activity against *L. monocytogenes* for the mature *pedA* than the $pedA^{Pre}$ precursor, while Van Reenen et al. (2003) expressed the native plantaricin 423 in *S. cerevisiae* L5366h. Both the pediocin PA-1 and plantaricin 423 expression systems used the yeast alcohol dehydrogenase I promoter ($ADH1_P$) and terminator ($ADH1_T$), resulting in low titres. More recently, Rossouw et al. (2024) expressed both native and codon-optimized forms of plantaricin 423 (PlaX) and mundticin ST4SA (MunX) in *S. cerevisiae* Y294 using *ENO1-MFα1* expression cassettes. The peptides were purified via HPLC, yielding 20.9 mg/L for MunX and 18.4 mg/L for Plax. These yields were 40.67% and 34.78% higher than those previously reported for *E. coli* expression (Vermeulen et al., 2020). Rossouw et al. (2025) also expressed the codon-optimized *E. faecium* enterocin A gene, *EntA_Opt*, in *S. cerevisiae* Y294, which yielded 50.93 mg/L and a minimum inhibitory concentration (MIC) of 192.74 nM against *L. monocytogenes* EDG-e.

The expression of bacteriocins by industrial strains of *S. cerevisiae* used in bioethanol fermentation could enable these strains to produce AMPs during fermentation. This concept was demonstrated by Rossouw et al., (2026) with an industrial *S. cerevisiae* strain coexpressing α-amylase, glucoamylase, and mundticin ST4SA. The resulting strain inhibited the growth of the natural LAB population in red sorghum fermentation and increased ethanol yields by 9% compared to the host strain without munst4sa. Although considerable progress has been made in producing recombinant bacteriocins in yeast (Rossouw et al., 2024, 2025), further optimization is needed to improve strain performance, expression, activity, stability, and peptide purification.

Despite the substantially higher peptide titers reported for *P. pastoris* compared to *S. cerevisiae*, the practical application of bacteriocin in industrial fermentations should be assessed more broadly. Recombinant protein production in *P. pastoris* frequently relies on inducible promoters, such as the *AOX1* promoter, which require methanol feeding or tightly controlled carbon-source shifts to initiate expression. These induction strategies introduce additional process complexity, safety considerations, and operational costs at an industrial scale. In contrast, *S. cerevisiae* usually employs constitutive expression systems that eliminate the need for inducer-dependent regulation, offering advantages in process integration and economic feasibility. The antimicrobial efficacy of bacteriocins depends on multiple context-specific factors, including the nature and concentration of contaminating bacteria, the susceptibility of dominant contaminants, and the peptide's MIC against target organisms. Industrial ethanol fermentations exhibit considerable variability in substrate characteristics and contaminant populations, precluding a universal peptide concentration that would be effective for bacterial control. While lower expression levels in *S. cerevisiae* may be a limitation under conditions of severe contamination, they may be adequate for bacteriocins with high potency and specificity toward dominant contaminants.

Strategic considerations for bacteriocin expression in *S. Cerevisiae*

A simplified view of practical recombinant gene expression begins with transcription of the genetic code in the nucleus, followed by translation by ribosomes in the cytoplasm, and ends with protein se-

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Table 1 Examples of AMPs recombinantly produced in *P. pastoris* (*K. phaffii*).

Antimicrobial peptide	Source	Expression*	Promotor	Vector	Yield	Antimicrobial activity	Reference
Abaecin	*Apis mellifera*	I	AOX1	pPIC9K	ND	Antibacterial	Luiz et al. (2017)
ABP-CM4	*B. mori*	I	AOX1	pPICZaA	ND	Antibacterial	Zhang et al. (2006)
ABP-dHC-cecropin A	*Hyphantriacunea*	I	AOX1	pPICZaA	21 mg/L	Antibacterial, antifungal	Sang et al. (2017)
Antifungal protein	*Aspergillus giganteus*	I	AOX1	pPICZaA	2.5 mg/L	Antifungal	López-Garcia et al. (2010)
Apidaecin	*A. mellifera*	I	AOX1	pPIC9K	418 mg/L	Antibacterial	Chen et al. (2017)
Apidaecin IA	*A. mellifera*	I	AOX1	pAOX1-HSA	>700 mg/L	Antibacterial	Cao et al. (2018)
Cathelicidin CAP18LL-37	Human	C	GAP	pGAPZ-E	ND	Antibacterial	Hong et al. (2007)
Cathelicidin CAP18LL-37	Human	I	AOX1	pPICZaA	ND	Antibacterial	Kim et al. (2009)
CecropinA(1–8)–magainin2(1–12) (CA-MA)	*Hyalophora cecropia*, *Xenopus laevis*	I	AOX1	pPICZ-A	22 mg/L	Antibacterial, antifungal	Jin et al. (2006)
Cecropin D	*B. mori*	C	GAP	pGAPZaA	485.2 mg/L	Antibacterial	Guo et al. (2012)
CecropinA(1–7)-thanatin(4–19)	*H. cecropia*, *Podisus maculiventris*	I	AOX1	pPICZaA	ND	Antibacterial	Liu et al. (2018)
CecP4	Pig intestinal parasite	I	AOX1	pPICZB	ND	Antibacterial	Song & Lee (2014)
Ch-Penaeidin (rCHP)	*Fenneropenaeus chinensis*	I	AOX1	pPIC9K	108 mg/L	Antibacterial, antifungal	Li et al. (2005)
Chromogranin A-derived CGA-N12 peptide	Human	I	AOX1	pPIC9	30 mg/L	ND	Li et al. (2020)
α-Defensin (HD5)	Human	I	AOX1	pPIC9K	165 mg/L	Antibacterial	Wang et al. (2009)
β-Defensin (msBD-1)	Sheep	I	AOX1	pPIC9K	35 mg/L	Antibacterial, antifungal	Zhao & Cao (2012)
β-Defensin 2 (hPAB-b)	Human	I	AOX1	pPIC9K	241 mg/L	Antibacterial	Chen et al. (2011)
β-Defensin 2	Porcine	I	AOX1	pPICZαA	383.7 mg/L	Antibacterial, antifungal	Peng et al. (2014)
Defensin (PaDef)	*Persea americana* var. *drymifolia*	I	AOX1	pPICZαA	79.6 mg/L	Antibacterial	Meng et al. (2017b)
Defensin (Pdc1)	Corn inbred line CO387	C	GAP	pGAPZaA	ND	Antifungal	Kant et al. (2009)
Defensin (Psd1)	*Pisum sativum*	I	AOX1	pPIC9	63 mg/L	Antifungal	Cabral et al. (2003)
Defensin (VrD1)	Mugbean	I	AOX1	pPIC9K	ND	Antifungal	Chen et al. (2004)
Defensin SPE10	*Pachyrrhizus erosus*	I	AOX1	pPIC9	7 mg/L	Antifungal	Song et al. (2005)
Enterocin L50 (EntL50A)	*E. faecium*	I	AOX1	pPICZαA	228.5 mg/g DCW	Antibacterial, antifungal	Basanta et al. (2010)
Enterocin L50 (EntL50B)	*E. faecium*	I	AOX1	pPICZαA	1 240 mg/g DCW	Antibacterial, antifungal	Basanta et al. (2010)
Enterocin P	*E. faecium*	I	AOX1	pPICZαA	22.8 mg/L	Antibacterial	Gutiérrez et al. (2005)

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Table 1 Continued

Antimicrobial peptide	Source	Expression*	Promotor	Vector	Yield	Antimicrobial activity	Reference
Fowlicidin-2	*Gallus gallus*	I	AOX1	pPICZαA	85.6 mg/L	Antibacterial	Xing et al. (2016)
Hepcidin-25	Human	I	AOX1	pPICZαA	1.9 mg/L	Antibacterial	Janakiraman et al. (2015)
Hispidalin	*Benincasa hispida*	I	NS.	pPICZαA	98.6 μg/ml	Antibacterial	Meng et al. (2019)
Human cathelicidin LL37	Human	I	AOX1	pPICZαA	ND	Antibacterial	Zhan et al. (2021)
Lactoferrampin fused with lactoferricin (LFA-LFC)	Bovine	I	AOX1	pPICZαA	ND	Antibacterial, antifungal	Tang et al. (2012)
Lactoferricin	Bovine	I	AOX1	pPICZαA	193.9 mg/L	Antibacterial	Cui et al. (2023)
Lactoferricin (LFcinC)	Camel	I	AOX1	pHILa	ND	Antibacterial, antifungal	Chahardooli et al. (2016)
Melittin	*A. mellifera*	I	AOX1	pPIC9	105 μg/L	Antibacterial	Moridi et al. (2020)
Plectasin (MP1106)	*Pseudoplectania nigrella*	I	AOX1	pPICZαA	8 321 mg/L	Antibacterial	Cao et al. (2015)
Plectasin (MP1102)	*P. nigrella*	C	GAP	GAP	807.4 mg/L	Antibacterial	Mao et al. (2015)
NZ17074	*Arenicola marina*	I	AOX1	pPICZαA	4.1 mg/L	Antibacterial, antifungal	Wang et al. (2014)
Plectasin-derived NZ2114	*P. nigrella*	I	AOX1	pPICZαA	2 390 mg/L	Antibacterial	Zhang et al. (2014)
Pediocin PA-1	*Pediococcus acidilactici*	I	AOX1	pPICZαA	25 μg/ml	Antibacterial, anti-cancer	Thu et al. (2024)
Plectasin	*P. nigrella*	I	AOX1	pPICZαA	748.63 mg/L	Antibacterial	Zhang et al. (2011)
Plectasin	*P. nigrella*	C	GAP	pGAPZ	370 mg/L	Antibacterial	Chen et al. (2016)
Pleurodicin	*Pleuronectes americanus*	I	AOX1	pPICZαA	10 μg/L	ND	Burrowes et al. (2005)
Porcine interferon-a (pIFN-a)	Porcine	I	AOX1	pPICZαA	ND	ND	Yu et al. (2010)
Protegrin-1	Porcine	I	AOX1	pPICZαA	40 mg/L	Antibacterial	Niu et al. (2015)
Snakin-1 (SN-1)	*Solanum tuberosum*	I	AOX1	pPIC9k	40 mg/L	Antibacterial, antifungal	Kuddus et al. (2016)

Note. *C = constitutive; I = induced; NS = not specified; ND = not determined.

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Table 2 Examples of AMPs recombinantly produced in *S. cerevisiae.*

Antimicrobial peptide	Source	Expression	Promotor	Vector	Yield	Antimicrobial activity	Reference
Cecropin P1	*A. suum*	I	T7	pYES2/CT-α factor	7.83 mg/L	Antibacterial, antiviral	Jiang et al. (2021)
Cecropin XJ	*B. mori*	I	GAL1	pYES2/CT-α factor	1.44 mg/L	Antibacterial	Xia et al. (2013)
Enterocin A	*E. faecium*	C	ENO1	pMR	85.77 mg/L	Antibacterial	Rossouw et al. (2025)
Enterocin L50A and L50B	*E. faecium*	I	GAL1	pYES2	0.56 mg/L	Antibacterial	Basanta et al. (2009)
Human β-defensin-1 (Hbd-1)	Human	C	ADH1	pVT103L	0.55 mg/L	Antibacterial	Cipáková & Hostinová (2005)
Mundticin ST4SA	*E. mundtii*	C	ENO1	pMR	20.9 mg/L	Antibacterial	Rossouw et al. (2024)
Pediocin PA-1	*P. acidilactici*	C	ADH1	pPRL2	ND	Antibacterial	Schoeman et al. (1999)
Penaeidins (Pen-2 and Pen-3a)	*Penaeus vannamei*	I	MFα1	pTG4812	ND	Antibacterial, antifungal	Destoumieux et al. (1999)
Plantaricin 423	*L. plantarum*	C	ADH1	pPRL2	N.D.	Antibacterial	Van Reenen et al. (2003)
Plantaricin 423	*L. plantarum*	C	ENO1	pMR	18.4 mg/L	Antibacterial	Rossouw et al. (2024)

Note. ∗C = constitutive; I = induced; ND = not determined.

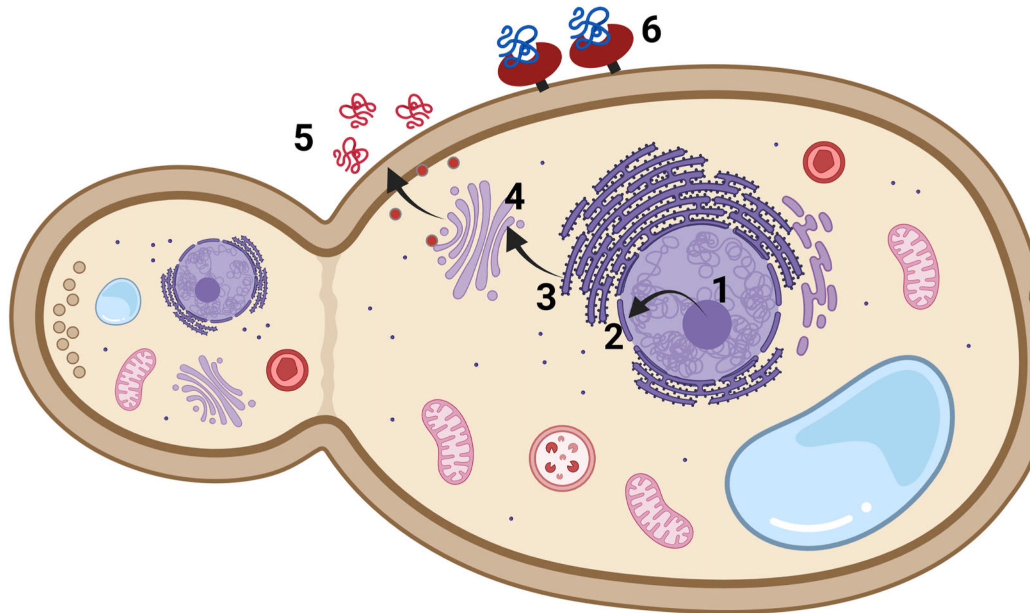

Fig. 7 Schematic overview of recombinant bacteriocin production in *S. cerevisiae*: (1), transcription in the nucleus; (2), translocation and translation by ribosomes; (3), secretion signal cleavage, folding, and initial glycosylation in the ER. (4), Transport to the Golgi apparatus, further glycosylation and packaging into membrane-bound vesicles for (5), secretion or (6), cell-surface display (created in https://BioRender.com).

cretion (Figure 7). However, each of these processes involves multiple steps and presents challenges that affect the efficiency of each stage and the final delivery of an active protein at high levels.

Several recombinant proteins have been successfully expressed in *S. cerevisiae*, offering a comprehensive understanding of the considerations and strategies available for various industrial applications (Den Haan et al., 2021). The following sections briefly outline strategies to improve the expression of recombinant bacteriocins in *S. cerevisiae* and are summarised in Figure 8.

Promotors

Different promoters can drive the transcription of recombinant AMP genes, depending on the host organism and the AMP's characteristics. Since antibacterial peptides may be toxic to *E. coli*, heterologous expression in this host often uses inducible promoters to first allow cell growth before initiating AMP production (Hoelscher et al., 2022). Mulder et al. (2015) found that a modified clavinin AMP expressed in *P. pastoris* with an inducible *AOX1* promoter was 1.5 times more abundant than with a constitutive *GAP1* promoter. However, inducible pro-

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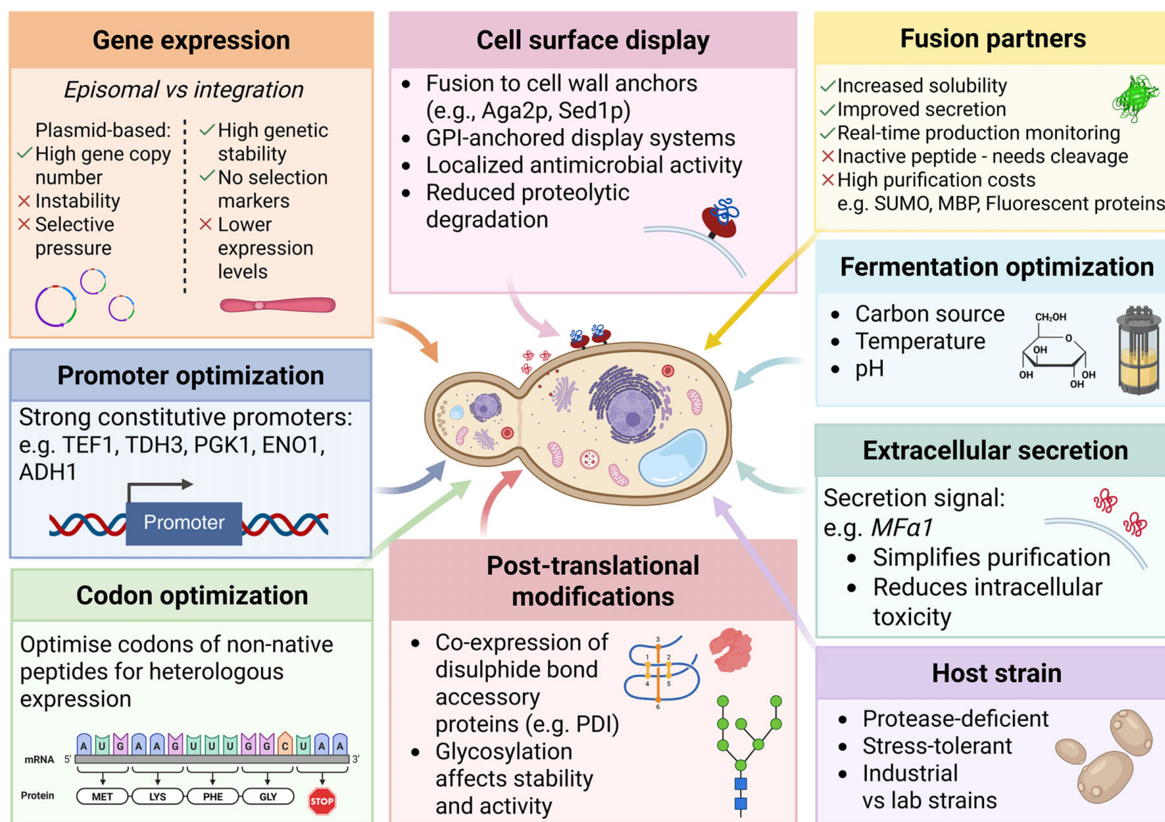

Fig. 8 Strategic considerations for bacteriocin production in *S. cerevisiae* for enhanced yield and activity (created in https://BioRender.com).

moters are less favoured for industrial-scale AMP production due to the high costs of inducers and the additional downstream processing required. Using a strong constitutive promoter and terminator enables continuous AMP expression and higher peptide yields. For instance, codon-optimized versions of *plaA* and *munST4SA* using the constitutive *ENO1* promoter and terminator produced 20.9 mg/L of MunX and 18.4 mg/L of PlaX in *S. cerevisiae* Y294 (Rossouw et al., 2024). Several other strong constitutive promoters can be used in *S. cerevisiae*, such as $TDH3_p$ and $TEF1_p$ (Den Haan et al., 2021) However, the choice of promoter should consider the type and/or concentration of carbon source and growth conditions (aerobic vs. anaerobic), as this could influence the expression levels of the promoter and subsequent production of peptide.

Codon optimization

Codon preference significantly influences gene expression levels and protein folding, making it a key factor in heterologous gene expression. Because of the redundancy in the genetic code, multiple nucleotide sequences can encode the same protein or peptide. The distribution of synonymous codons in a genome is uneven, with different genomes exhibiting different codon usage frequencies (Wu et al., 2007), leading to codon usage bias that affects translation efficiency. To enhance foreign protein expression in a recombinant host, codon optimisation techniques replace rare codons with more common ones that the host uses. Various indices, based on codon usage bias, can predict individual gene expression levels. Nevertheless, multiple factors influence codon choice, and identifying the optimal synthetic gene sequence for *S. cerevisiae* often involves trial and error (Parret et al., 2016).

Although codon optimisation can enhance gene expression levels in *S. cerevisiae*, it can influence the protein's tertiary structure (Yadava & Ockenhouse, 2003). Nguyen et al. (2020) described an *S. cerevisiae* strain that expressed codon-optimized pediocin PA-1 via a cell-surface display, but it lost activity against previously susceptible *L. monocytogenes*, *E. faecalis* and *S. aureus*. In contrast, Rossouw et al. (2024) successfully expressed and secreted both native and codon-optimized versions of genes for plantaricin 423 and mundticin. The codon-optimized mundticin gene demonstrated an eightfold higher activity relative to the native gene, whereas activity levels for native and optimized plantaricin 423 remained similar. These differences may relate to the codon bias index, which increased from 0.05 for munST4SA to 0.59 for *munST4SA_Opt*, compared with a rise from 0.14 for *plaA* to 0.23 for *plaA_Opt*.

Episomal plasmids versus genomic integration

AMP expression can be achieved by cloning the gene into either an episomal vector or through genomic integration (Den Haan et al., 2021). Typically, gene copy number is expected to correlate with expression level. Yeast plasmids used for this purpose, whether integrative or episomal, contain an auxotrophic selection marker (*LEU2, URA3, HIS3, TRP1*, or *LYS2*). The yeast-integrating plasmid inserts a single copy of the target gene into the genome (Jensen et al., 2014),

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while the yeast episomal plasmid contains a 2μ origin, resulting in 10–40 copies with low stability (Hohnholz et al., 2017). The need to sustain expression vectors under selective conditions limits the large-scale use of these systems.

An alternative method to achieve high expression levels in *S. cerevisiae* is delta-integration, which involves inserting multiple copies of a gene into δ-sequences on the yeast chromosome (Sakai et al., 1990). However, integration into δ-sites is random; the locations and quantities of integrated gene cassettes are unknown and differ among transformants, necessitating extensive screening. Nonetheless, this approach allows for the stable incorporation of multiple gene copies and does not require selective pressure to maintain the recombinant gene cassette.

A challenge in marker-assisted homologous recombination is the limited availability of selectable markers, the necessity for specific growth conditions to sustain the recombinant gene—especially in lab strains—and the restricted use of the strain on a commercial scale if an antibiotic marker becomes part of the yeast genome. The CRISPR-Cas system (clustered regularly interspaced short palindromic repeats-CRISPR-associated proteins) has emerged as a highly effective gene editing tool for *S. cerevisiae* (Wu et al., 2025) without the need for selectable markers or special growth conditions. Additionally, marker-less approaches, such as recyclable markers, e.g., the dominant *amdSYM* cassette (Solis-Escalante et al., 2013), can be used for strain engineering. The counterselection process to remove this marker provides an effective method for developing industrial *S. cerevisiae* strains.

Secretion signals

Peptide production can be enhanced by employing a secretion signal that directs the peptide to the extracellular matrix. This facilitates purification, shields the peptide from intracellular protease degradation, and reduces misfolding, as chaperone proteins assist proper folding and are secreted (Kleiner-Grote et al., 2018). While most AMPs have natural secretion systems in their hosts, recombinant hosts lacking these systems may result in peptide build-up inside the cell. The secretion of recombinant AMPs in yeast mainly relies on fusing the mating factor-alpha (MFα1) secretion signal to the AMP of interest. The MFα1 secretion signal includes a Kex2 cleavage site (KR) and two Ste13 protease sites (EAEA), enabling complete removal of the recombinant peptide's N-terminal end. In *S. cerevisiae*, the MFα1 pre-sequence is cleaved in the ER; the pro-sequence is cut by Kex2 protease in the Golgi apparatus, before the Ste13 protease removes the remaining amino acids at the N-terminus (Dalvie et al., 2022).

Studies have confirmed that AMPs can be effectively secreted using the *Trichoderma reesei* xylanase 2 secretion (XYNSEC) signal (Chahardooli et al., 2016; Gutiérrez et al., 2005; Jin et al., 2006; López-Garcia et al., 2010). However, Rossouw et al. (2024) observed significantly larger inhibition zones when expressing plantaricin 423 and mundticin ST4SA with the MFα1 secretion signal in *S. cerevisiae*, while the supernatant from the XYNSEC variants showed no activity. The XYNSEC secretion signal contains only a Kex2 site and lacks Ste13 proteases, which can result in incomplete Kex2 cleavage and protein aggregation, possibly facilitated by the remaining Glu-Ala repeats still attached to the N-terminus of the recombinant protein. N-terminal extensions may lead to improper protein folding (maturation) and ineffective secretion of the peptides.

Cell-surface display

Cell-surface display of proteins enables functional recombinant proteins to be presented on the cell surface, with multiple successes reported for cellulases and amylases expressed in *S. cerevisiae* (Zhang et al., 2022a). Nguyen et al. (2020) successfully expressed pediocin PA-1 in *S. cerevisiae*, achieving a yield of 4.75 g dry cell weight per litre and demonstrating effective inhibition of *Shigella boydii* and *Shigella flexneri*. Cell-surface display technology sidesteps issues with enzyme purification, substrate transport, and enzyme instability (Zhang et al., 2022a). Nonetheless, it faces several challenges, including low heterologous protein production, leading to minimal or no activity; reduced activity after recycling; the need for improved surface space utilisation in yeast cells; ensuring that engineered strains are suitable for industrial-scale production; and limited data on their tolerance to inhibitory compounds.

Since some AMPs can kill or inhibit their microbial host, tethering these peptides to the cell wall reduces toxicity by limiting direct interaction with the host's cell membrane. Furthermore, these anchored peptides can be protected from protease degradation under industrial fermentation conditions, thereby extending their antimicrobial activity over a longer period. An additional benefit of this technique is simplified downstream processing; instead of purifying the peptides from the yeast supernatant, the whole cell can serve as the antimicrobial product, thereby avoiding purification costs. Disadvantages of this technique include poor control over enzyme ratios, which can negatively affect the optimisation of enzyme cocktails when more than one peptide is required, and limited cell-surface space (Zhang et al., 2022).

Post-translational modifications

Extracellular activity can be affected by post-translational modifications of peptides, such as glycosylation and disulphide bond formation. Class IIa bacteriocins typically undergo few modifications, mainly disulphide bonds to varying degrees (Liang et al., 2025). For example, mature mundticin ST4SA has one disulphide bond, whereas plantaricin 423 and enterocin A each have two bonds. Native bacteriocin operons usually include accessory proteins that facilitate disulphide bond formation; however, these genes are absent in the enterocin A operon. Rossouw et al. (2025) found peptide concentrations for EntA_Opt to be twice as high as for PlaX_Opt and MunX_Opt produced in *S. cerevisiae*, yet plantaricin 423 showed significantly lower activity. Nano-LC-MS/MS analysis indicated a greater proportion of EntA_Opt peptides with correct disulphide bonds compared to PlaX_Opt. This highlights the crucial role of post-translational modifications, especially correct disulphide bond formation, in peptide function and stability. Coexpressing disulphide bond accessory proteins in *S. cerevisiae* could promote disulphide bond formation and improve peptide activity

Glycosylation entails the addition of a sugar group to a protein or peptide, and is classified as N-, O-, C-, or S-glycosylation (Bellavita et al., 2023). N-linked glycosylation involves the addition of a 14-sugar glycan tree to the N-X-S or N-X-T recognition sequence of the asparagine residue by oligosaccharyl transferase (OST). O-linked glycosylation is performed by O-mannosyltransferases (PMTs), which add a single mannose to serine or threonine residues in the ER. Additional mannoses can be attached later in the Golgi apparatus (Hou et al., 2012). The specific type of glycosylation depends on factors such as glycan composition, glycosidic linkage, structure, and length. The extent and type of glycosylation vary between strains and proteins. For instance,

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predictions suggest that recombinant enterocin A is likely to be O-glycosylated, whereas the mundticin ST4SA variant may undergo N-glycosylation (Rossouw et al., 2025).

Glycosylation can either enhance or diminish the activity of AMPs, as added sugar units can cause conformational changes in the peptide backbone. It impacts the peptide's hydrophobicity and overall amphiphilicity, which subsequently influence its interactions with and insertion into bacterial anionic membranes. Glycosylation can also alter the peptides' bacterial targeting specificity (Bellavita et al., 2023). Grimsey et al. (2020) observed a 2 to 16-fold reduction in antimicrobial activity among different glycosylated AMP species, with various sugar-peptide combinations producing diverse effects on activity. Huang et al. (2013) reported that glycosylation of the lysostaphin peptide enabled it to bind but not lyse *S. aureus*. The peptide's lytic activity was restored after replacing the amino acid glycosylation site. Additionally, glycosylation can improve peptide stability by shielding it from proteases or induce a conformational change, making it more compact or protected (Bednarska et al., 2017). Glycosylation predictors such as GlycoEP (https://webs.iiitd.edu.in/raghava/glycoep/) are useful tools to determine potential glycosylation of bacteriocins in heterologous hosts, but analytical and stability testing should be performed to confirm such predictions. Understanding peptide glycosylation patterns and their effects on biological activity and stability would enable the rational engineering of glycosylation to enhance peptide performance.

Fusion proteins

An alternative approach is to attach a carrier protein to the AMP of interest. This carrier inactivates the AMP, preventing harm to the host and shielding it from protease degradation (Li, 2011). Fusion protein partners can also promote proper folding and disulphide bond formation and enhance protein solubility (Ingham & Moore, 2007). Common fusion partners for bacterial expression of AMPS include glutathione S-transferase, maltose-binding protein, thioredoxin, SUMO, and fluorescent proteins (Mesa-Pereira et al., 2018). Since the fused AMP is inactive, the fusion partner must be enzymatically cleaved, which can result in low yields and high costs, rendering it unsuitable for large-scale production.

Alternative *S. cerevisiae* host strains

Enhancing AMPs production in *S. cerevisiae* could be achieved by using more robust strains that are better equipped to withstand the harsh industrial conditions of large-scale manufacturing. Industrial *S. cerevisiae* strains offer several advantages: they grow more quickly and are more tolerant of higher temperatures, lower pH, and higher ethanol concentrations. Additionally, industrial yeast can thrive on less costly media or even on industrial waste, thereby reducing the expenses associated with recombinant peptide production. Nevertheless, many technical obstacles may arise when developing industrial strains for commercial use, such as low-transformation efficiencies and copy numbers during gene integration (Den Haan et al., 2023). Different host strains also exhibit unique glycosylation profiles, and gene integration efficiency can vary due to strain-specific differences in genome structure and DNA repair pathways. Rossouw et al., (2026) reported mundticin ST4SA expression in the *S. cerevisiae* Y294 laboratory and Ethanol Red™ industrial strains, resulting in varying activity levels linked to different glycosylation profiles. Additionally, gene expression varied between plasmid-based and integrated forms, aligning with differences in copy number.

Coexpression with hydrolytic enzymes

Producing bioethanol from starch involves considerable costs, mainly due to the high-quality raw material, the use of starch-hydrolysing enzymes, heating processes, and chemicals like lime, caustic soda, and sulphuric acid to maintain pH levels (Joyia et al., 2024). The process requires precise control because fermentation temperatures above 35 °C and ethanol levels over 20% (v/v) can impact the growth of *S. cerevisiae* cells. A more advanced approach was to design an *S. cerevisiae* strain that hydrolyses starch and ferments the sugars to ethanol, known as consolidated bioprocessing (CBP) (Favaro et al., 2015). Cripwell et al. (2019) observed a significant boost in bioethanol yield using a recombinant *S. cerevisiae* expressing a codon-optimized *Talaromyces emersonii* glucoamylase and α-amylase. Additionally, Rossouw et al., (2026) developed an industrial amylolytic *S. cerevisiae* ER T1 strain (based on Ethanol Red™) that coexpresses α-amylase, glucoamylase, and recombinant mundticin ST4SA, which increased ethanol production by 4.27 g/L compared to ER T1 in non-sterile red sorghum fermentation without antibiotics.

The production of heterologous proteins in yeast requires substantial cellular resources for their synthesis, folding, and transport and can impose a metabolic burden through several underlying mechanisms (reviewed by Kastberg et al., 2022). A metabolic burden may result in reduced growth rates, decreased biomass, and often reduced ethanol yields. Furthermore, the production of abnormally high protein levels (as often reported in genetically engineered strains), may overwhelm the ER folding machinery and lead to an increase in reactive oxygen species. Given their small size, AMPs are typically assumed to require relatively modest cellular resources for biosynthesis, suggesting that their heterologous expression in *S. cerevisiae* should not inherently result in significant metabolic stress. Rossouw et al. (2024) observed no significant impact on the biomass of *S. cerevisiae* strains producing plantaricin 423 and mundticin ST4SA, indicating that the growth of the yeast was not affected by peptide production. Furthermore, a bacteriocin-producing CBP strain coproducing two amylases and enterocin A, exhibited growth rates similar to those of the control strain lacking amylases or bacteriocin (Rossouw et al., 2026).

Outlook and Conclusions

The potential of bacteriocin-producing *S. cerevisiae* offers innovative, sustainable, and effective solutions for managing bacterial contamination in bioethanol production. The inherent stability of bacteriocins makes them suitable for yeast hosts with high resilience and for long-term fermentations. Importantly, bacteriocins possess probiotic and non-toxic properties, offering a safer alternative to AMPs that may require extensive toxicity testing. Their application could also support existing antimicrobial treatments, potentially reducing overall reliance on antibiotics in industrial fermentations.

Although bacteriocins are small and can be chemically synthesised, their high production costs limit their feasibility for large-scale commercial application. Harvesting AMPs from other classes of natural sources, such as higher eukaryotic hosts, can be difficult and time-consuming. The purification of class IIa bacteriocins remains challenging due to low yields, and purified peptides are generally not used

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commercially. Instead, a bacteriocin-producing organism, crude bacterial fermentate or a partially purified peptide is applied.

Heterologous expression may be the most cost-effective method to produce pure bacteriocins. Although several recombinant AMPs have been expressed in *P. pastoris* (*K. phaffii*), limitations in the expression of some peptides have also been reported. *Saccharomyces cerevisiae* provides a more suitable expression system, along with a variety of molecular tools, for producing class IIa bacteriocins, since they do not require complex post-translational modifications and are inactive against *S. cerevisiae*. Class IIa bacteriocins are particularly suitable for targeting LAB, the main contaminants in bioethanol fermentations that cause stuck fermentations and reduce ethanol yields. Therefore, in addition to the potential to produce bacteriocins on a larger scale, developing bactericidal strains of *S. cerevisiae* could enhance bacteriocin applications in the biofuels sector. In this context, yeast can be used directly in bioethanol fermentations to control contaminating bacteria without requiring peptide purification.

Several genetically modified yeast strains are used for commercial ethanol production (da Silva Fernandes et al., 2022), and it is expected that more processes will adopt engineered strains to boost ethanol yields and improve fermentation performance. However, it is acknowledged that industrial adoption of genetically modified microorganisms remains limited by regulatory constraints and market barriers. In some countries, regulatory frameworks for the use of genetically modified organisms in fermentations require their use in contained settings, while other fermentation products (aside from ethanol) are regulated by separate food/feed additive laws (Lensch et al., 2022). Provided that the genetically modified strains are removed from the final product, regulatory barriers may not apply, at least in some countries.

Future research should explore the coexpression of multiple bacteriocins or the development of bacteriocin 'cocktails' that target various LAB species, considering the diversity of immunity protein classes, which can affect strain sensitivity. The specific application should inform strain design, as different fermentation substrates and process conditions will require tailored approaches. Integrating this strategy into a circular bioeconomy model and using low-cost or waste-derived substrates could further improve the process's sustainability.

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Author Contributions

Michelle Rossouw: Conceptualization, Writing – original draft, review & editing. **Bianca J. Campbell**: Writing – original draft, review & editing. **Leon M.T. Dicks:** Conceptualization, Writing – review & editing. **Rosemary A. Cripwell, Marinda Viljoen-Bloom:** Conceptualization, Resources, Supervision, Writing – review & editing

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Conflicts of interest

None declared.

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