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Combined use of bacteriocins and bacteriophages as food biopreservatives. A review

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ABSTRACT

Throughout history, humans have consistently developed strategies to prevent food-associated illnesses. However, despite our multiple technological advances, food safety is still an issue of concern. Moreover, there is a demand for gaining access to less processed and naturally preserved food. Food biopreservation, understood as the use of natural antimicrobials already present in food with a long history of safe consumption, is seen as a plausible strategy to reduce the intensity of current preservation technologies (e.g., presence of chemically synthesized food preservatives). In that sense, the combined use of several antimicrobial strategies, known as hurdle technology, has been often chosen as a means to improve the efficacy of food biopreservation. This review intends to summarize the most recent examples of the combined use of bacteriocins and bacteriophages to extend food shelf-life and reduce the risks associated with the presence of foodborne bacteria along the food chain. However, while the efficacy of bacteriocins has been extensively documented, bacteriophages have only started to be assessed as potential food biopreservatives more recently. Within this context, we would like to consider whether these two types of natural antimicrobials would help each other to overcome bottlenecks in food biopreservation.

1. Introduction

Even in the 21st century, foodborne diseases remain one of the most serious health concerns worldwide. According to the last available EFSA report, 5175 food-borne outbreaks, involving 49,463 cases which include 2859 hospitalizations and 60 deaths, were reported in the EU during 2019. The number of outbreaks of the most common pathogens such as *Campylobacter* and *Salmonella* has been stable in the last 5 years. However, others such as *Listeria monocytogenes* or Shiga toxin-producing *Escherichia coli* have been on the rise. This suggests that the control of food pathogens is still an important challenge for our society (European Food Safety Authority and European Centre for Disease Prevention and Control, 2021).

These undesirable microorganisms may be present in food products, processing facilities or manufacturing environments and could be transmitted all over the food chain. Besides the presence of planktonic cells, biofilms formed on manufacturing surfaces represent one of the main sources of contamination, and are very difficult to eradicate because of their extraordinary resistance to traditional disinfection methods (Duraisamy et al., 2020; Gutiérrez et al., 2016). In addition to this, the growing demand for ready-to-eat, fresh-cut and minimally processed food, as well as the trend towards 'clean label' and more natural food pushes the food industry to apply strategies aimed at reducing the use of additives and other traditional food preservation methods (Asioli et al., 2017). In this context, biopreservation, defined as the rational use of antimicrobials that are naturally present in food with a long history of safe use, is gaining importance due to their specific properties. More explicitly, the use of microorganisms or their metabolites for the preservation of food is expected to impact minimally the nutritional and sensory properties while extending shelf-life (Gálvez et al., 2010).

Within the different possibilities available in the field of biopreservation, bacteriocins, the antimicrobial peptides synthesized by bacteria, and bacteriophages, the viruses that infect bacteria, are without a doubt promising candidates. Indeed, both of them are naturally present in food and their role as potent antimicrobials has been

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well documented (García et al., 2010b; Martínez et al., 2019; Mills et al., 2017). Bacteriocin and phage preparations are commercially available and applied according to country regulations. In Europe, only the bacteriocin nisin is authorized as an additive (Younes et al., 2017), but protective cultures, many of them being bacteriocin producers, are commercialized as shelf-life extenders in meat and dairy products. On the other hand, phages have been evaluated by EFSA, who concluded that they are safe for consumers and the environment, although each phage (or phage cocktail) intended to be applied in food must be assessed on a case-by-case basis due to the complexity of host: phage interactions (Ricci et al., 2017).

Nevertheless, the semi-solid state and the physico-chemical properties of certain foods may seriously compromise the in situ efficacy of these natural antimicrobials. Moreover, their spectrum of activity, particularly in the case of phages, may not cover all the potential bacterial targets. To overcome these limitations, their use in combination with other hurdles that curtail bacterial growth, i.e., hurdle technology, should be carefully considered. This approach is based on the combination of different processes to ensure stable and safe food (Leistner, 1992) and, since the introduction of hurdle technology in the food sector, it has been successfully applied to extend the shelf-life of different types of food. In this review, we summarize the current-state-of-the-art in food biopreservation through the combined use of bacteriocins and bacteriophages. Such a strategy will fully realize the potential of these antimicrobials in the context of hurdle technology and, hopefully, help food stakeholders to meet consumers' demands for minimally processed food without compromising food safety.

2. Antimicrobial peptides produced by bacteria: the bacteriocins

Bacteriocins are ribosomally synthesized antimicrobial peptides or proteins produced and secreted by bacteria. Initially discovered in Gram-negative bacteria, the isolation of nisin, the first bacteriocin produced by the Gram-positive bacterium *Lactococcus lactis*, along with its prompt application in food to prevent the late blowing defect in ripened cheese, boosted the quest for other bacteriocins with similar or improved antimicrobial activities. As a result, hundreds of new bacteriocins have been identified since then, with many being produced by lactic acid bacteria due to the widespread use of these bacteria in food fermentations and the role of their bacteriocins in preventing food spoilage and contamination with foodborne pathogens (López-Cuellar et al., 2016; Trejo-González et al., 2021). Moreover, comprehensive mining of diverse microbiomes by metagenomics is further increasing the bacteriocin portfolio (Garcia-Gutierrez et al., 2019; Tracanna et al., 2017; Zheng et al., 2015). Consequently, their molecular structures and modes of action are highly diverse (Acedo et al., 2018; Alvarez-Sieiro et al., 2016; Cotter et al., 2005). Class I bacteriocins are post-translationally modified peptides. The modifications comprise the presence of dehydrated amino acids, lanthionine and/or β-methyl lanthionine in the lanthipeptides with antibiotic activity (or lantibiotics, e.g. nisin), head-to-tail cyclized peptides such as enterocin AS-48, glycosylation (e.g. glycocin F), and further modifications that are introduced into a core pre-peptide which can be classified according to the modifying enzymes, as recently proposed (Montalbán-López et al., 2021). Class II are unmodified peptides which can be further subdivided into subclasses: class IIa includes peptides with potent anti-*Listeria* activity (e.g., pediocin PA-1); class IIb with two peptides displaying synergistic activity (e.g., lactococcin G); class IIc, leaderless bacteriocins generally with a broad spectrum of activity (e.g., enterocin L50); and class IId that encompasses other atypical unmodified peptides such as Lcn972. High-molecular weight antimicrobial proteins with either lytic activity (e.g., lysostaphin), non-lytic (e.g., colicins) and phage tail-like multiprotein complexes are regarded as class III bacteriocins. This structural diversity can be further expanded by multiple protein engineering possibilities (Field et al., 2015).

In line with their structural diversity, bacteriocin killing activities are also highly diverse although most of them target the cell envelope [Fig. 1]. Based on their cationic and amphiphilic nature, many bacteriocins are able to form pores in the cytoplasmic membrane of susceptible bacteria, disrupting the proton motive force and causing cell death (Vasilchenko and Valyshev, 2019). While membrane insertion is prompted by structural changes upon contact with bacterial membranes, as described for the circular enterocin AS-48 (Cebrián et al., 2015), pore formation may be facilitated by the specific interaction with docking molecules or receptors. Nisin and related lantibiotics bind with high affinity to the cell wall precursor lipid II (Brötz et al., 1998). Thus, two modes of action, i.e. pore formation and inhibition of cell wall biosynthesis, are combined within the same molecule for potent antimicrobial activity (Wiedemann et al., 2001). Pediocin-like and other class II bacteriocins such as lactococcin A make use of the mannose-PTS transporter as receptor (Diep et al., 2007), whereas other membrane proteins have been linked to bacteriocin activity based on the observation of a resistant phenotype when their genes are knocked-out in otherwise susceptible strains (Kjos et al., 2014; Miljkovic et al., 2016). Other bacteriocins only inhibit cell wall biosynthesis without pore formation (e.g. mersacidin, Lcn972) or act as peptidoglycan hydrolases (Martínez et al., 2020; Roces et al., 2012). Several microcins and colicins have intracellular targets and may inhibit transcription, translation and DNA replication (Telhig et al., 2020).

Resistance to bacteriocins is often achieved by adaptive responses that transiently modify the cell envelope, limiting the initial bacteriocin-cell interactions by changes in membrane fluidity, reduction of the negative cell surface charge, production of exopolysaccharides and/or suppression/mutation of non-essential bacteriocin receptors (Bastos Mdo et al., 2015; Draper et al., 2015). Unfortunately, activation of efflux pumps has also been involved in bacteriocin resistance (Campelo et al., 2020; Tymoszewska et al., 2021), a worrying risk of cross-resistance to clinical antibiotics. Therefore, bacteriocin resistance should be closely monitored.

The application of bacteriocins as food biopreservatives as well as the use of protective cultures to extend the shelf-life of dairy products, processed meat, vegetables and beverages have been comprehensively reviewed elsewhere (García et al., 2010b; Ibarra-Sánchez et al., 2020; Johnson et al., 2018). Class I and class II bacteriocins are often thermostable, active in a wide range of pH, sensitive to proteases in the gut, able to inhibit relevant foodborne pathogenic and spoilage bacteria such as *L. monocytogenes*, *Staphylococcus aureus* and *Clostridium tyrobutyricum,* among others, and also active against biofilms. Class III bacteriocins are heat labile and many display a narrow spectrum of inhibition, thus limiting their use as food biopreservatives. There is also an increasing number of studies addressing bacteriocin toxicity and their fate once consumed, important prerequisites for approval either as food preservatives or therapeutic antimicrobials (Benítez-Chao et al., 2021). However, the effectiveness of bacteriocins in food matrices is often challenged by their hydrophobic and cationic nature that implies poor solubility, limited diffusion and irregular partitioning due to interactions with food components [Fig. 2]. Moreover, bacteriocin susceptibility is strain-dependent and tolerance is highly variable within the target species (Ennahar et al., 2000). In order to overcome these limitations, innovative solutions embrace (nano-)encapsulation, active packaging, protein bioengineering and, above all, hurdle technology including bacteriocin-phage combinations as described in this review [Fig. 2].

3. The enemies of bacteria: the bacteriophages

Bacteriophages (or phages) are viruses that infect bacteria and represent the most abundant biological entities on Earth. Soon after their discovery in the early 20th century, d'Herelle proposed their application for the treatment of infectious diseases caused by bacteria (d'Hérelle and Smith, 1930). After all, as the old proverb says, “the enemy of my enemy is my friend”. This strategy, known as phage

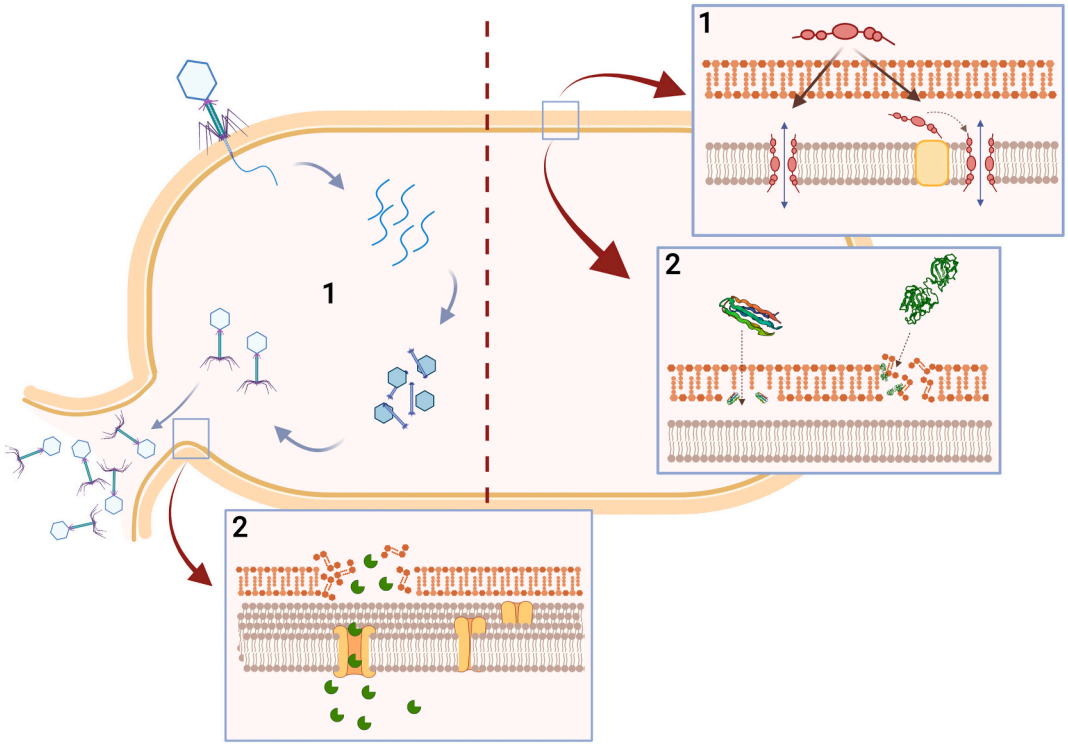

Fig. 1. Mode of action of bacteriophages (left) and bacteriocins (right). (1) The lytic cycle of bacteriophages in the host bacteria is indicated, including the last step of lysis carried out by holin and endolysin (2). Interaction between bacteriocins and the cell envelope leads to (1) pore formation in the cellular membrane or (2) inhibition of peptidoglycan synthesis or hydrolysis. Figure created with BioRender.com.

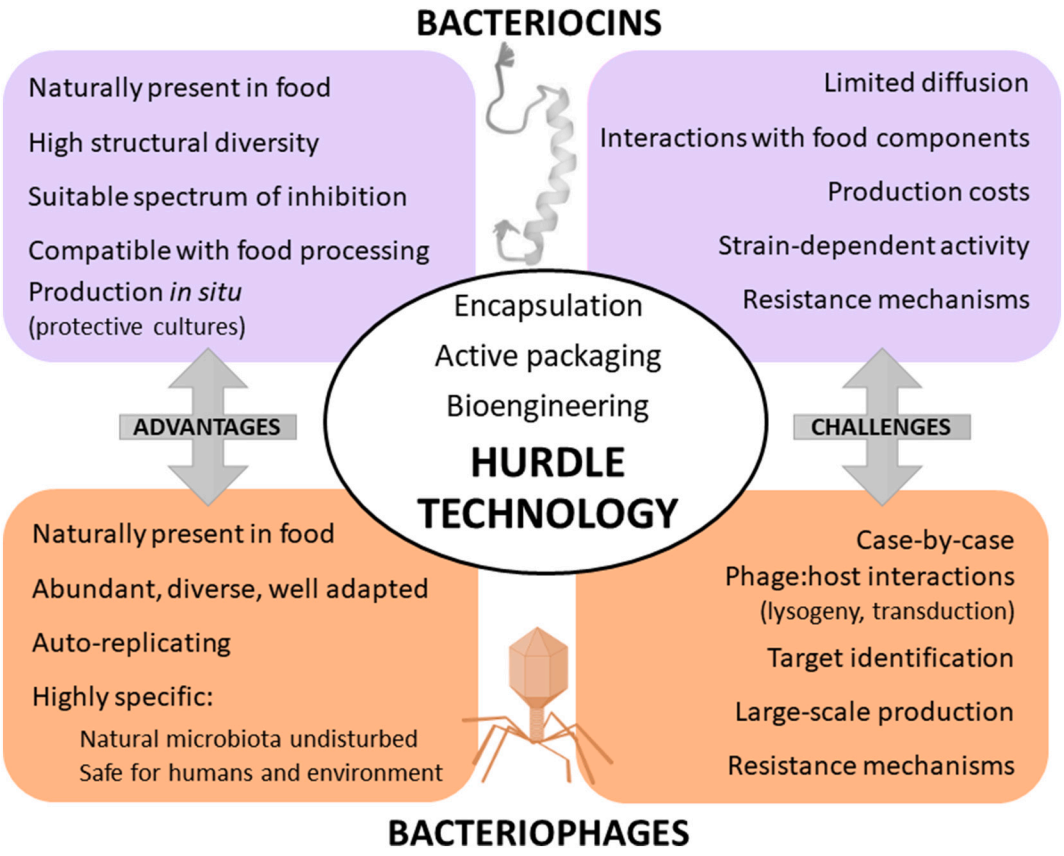

Fig. 2. Advantages and challenges of the use of bacteriocins (purple, up) and bacteriophages (orange, down) as food biopreservatives. Strategies (white, center) to improve their antimicrobial properties are also indicated.

therapy, was, however, soon displaced by antibiotics until, more recently, the interest in using bacteriophages as antimicrobials is making a comeback worldwide due to the rise of antibiotic resistance. Moreover, phage application is no longer relegated to the clinic, and is now proposed as a viable option to combat unwanted bacteria in different fields, including veterinary medicine and the food industry (Fernández et al., 2018; O'Sullivan et al., 2019).

The antimicrobial activity of phages is a consequence of their parasitic lifestyle, in particular, their development by the lytic cycle [Fig. 1]. During the first steps of this process, the virus takes over the host cell and uses its machinery to make copies of itself. Then, towards the end of the cycle, the newly formed viral particles are released due to lysis of the infected cell thanks to the activity of phage-encoded proteins with muranolytic activity, the so-called endolysins. This ability turns these simple entities, consisting only of a few proteins and a small genome, into very effective "bacterial killers".

Among the most notable advantages of bacteriophages as antimicrobials [Fig. 2], it is worth mentioning that they are very host-specific, which makes them innocuous not only for humans, animals, and plants, but also for non-target bacteria, including the normal microbiota and microorganisms involved in food production. Additionally, if there is a suitable host, their post-application levels will increase, instead of decreasing as is the case for other types of compounds. Moreover, due to their distinct mode of action, phages are effective against many antibiotic- and disinfectant-resistant bacteria.

Implementation of phage-based biocontrol strategies in food is also facing several challenges, mostly based on the complexity of the phage: host interactions which should be studied on a case-by-case basis (Fernández et al., 2018; Lewis and Hill, 2020). Identification of the target pathogen is a prerequisite for the application of phage-based products, precisely because of their specificity. Also, there is always a risk of phage resistance development, even though it is generally not as transferable to other microorganisms as antibiotic resistance markers. These two drawbacks can nevertheless be somewhat thwarted by using phage cocktails (a mix of different phages) instead of single-phage preparations (Chan et al., 2013). Another caveat is that some phages may actually promote antibiotic resistance gene transfer by releasing bacterial DNA or by transducing events (Keen et al., 2017) and, as a result, should not be used for biocontrol applications.

Besides whole bacteriophages, some phage proteins are also promising antimicrobials that can be used to avoid food contamination. Indeed, endolysins, lytic proteins responsible for degrading the bacterial cell wall peptidoglycan at the end of the lytic cycle, have been studied since the beginning of the millennium as a novel antibacterial strategy, and are frequently referred to as enzybiotics (Nelson et al., 2001). This application is based on the use of these proteins as exolysins, which will degrade the cell walls from without, i.e., when added exogenously, leading to cell lysis [Fig. 2]. This effect is relatively easy to accomplish in the case of Gram-positive bacteria, whose peptidoglycan is more accessible from outside the cell. In contrast, the outer membrane of Gram-negative bacteria makes lysis from without a lot more difficult. In order to circumvent that, several strategies have been developed, including the combined use of endolysins with chelating agents (Murray et al., 2021), or the design of artilysins, endolysins fused with a cationic peptide (Briers et al., 2014), and innolysins, fusion proteins consisting of a lytic domain and a phage receptor binding protein (Zampara et al., 2020). Also, endolysins from phages infecting Gram-positive bacteria (and some from those infecting Gram-negative bacteria) present a modular structure consisting of one or more enzymatic catalytic domains and a cell wall binding domain (CBD) (Schmelcher et al., 2012). This structure makes it possible to design new chimeric proteins via domain shuffling that frequently display an improvement in lytic activity compared to their "parent" proteins (Gutiérrez et al., 2018).

Despite sharing some common advantages with phages, it is important to highlight that endolysins can exhibit a narrow or broad host specificity, in contrast to the high specificity of phages (Schmelcher et al., 2012). Additionally, phage lytic proteins exert a rapid bactericidal action, do not easily select for resistant variants, and they are active against persister cells (Gondil et al., 2020). However, unlike phages, endolysins do not increase in number during treatment or remain active for a long time, being also very unstable depending on the environmental conditions (Gutiérrez et al., 2020). Because of their instability, food treatments based on endolysins would require repeated dosing to prevent cell regrowth. One strategy that can help with the short-lived activity of lytic proteins is their combination with other, more stable antimicrobials, such as antibiotics (Daniel et al., 2010), disinfectants or even bacteriophages (Duarte et al., 2021).

Overall, bacteriophages and their derived proteins represent a promising strategy for decontamination along the food chain. However, it is important to emphasize the importance of exploring their combined use with other compounds with the aim of overcoming shortcomings, such as those concerning the development of bacterial resistance to phages or the low stability of lytic proteins.

4. Combined use of bacteriocins and bacteriophages: examples from the literature

The combined use of bacteriocins, bacteriophages and phage-encoded proteins, such as endolysins, as food biopreservatives has been the subject of several studies, the results of which are summarized below and in Table 1, classified by the target bacterium.

4.1. *Listeria monocytogenes*

Not surprisingly, *L. monocytogenes* is the most common target reported in the literature [Table 1]. Although cases have remained stable after a long time of increase, its frequent presence in different kinds of foods, as well as the high fatality of the disease it causes, especially in at risk groups (the elderly, immunocompromised people, pregnant women and children) explain the focus on this pathogen (European Food Safety Authority and European Centre for Disease Prevention and Control, 2021).

Several bacteriocin-bacteriophage combinations have been applied in different food matrices with diverse results. The combination of nisin with several *Listeria* phages is the most common and, in the majority of cases, synergy between these two antimicrobials has been observed.

To the best of our knowledge, the first attempt was made by Leverentz et al. in 2003. Here, two different phage mixtures (LM-103 and LMP-102) containing 14 and 6 different lytic *Listeria* phages were combined with Nisaplin, a standardized commercial nisin preparation, and applied to fresh-cut apple and honeydew melon slices. Fruit samples were inoculated with *L. monocytogenes*, using a suspension of 5×10^5 cfu/mL and bacterial counts were determined for up to 7 days of storage at 10 °C. The observed bacterial population reduction of 5.8 and 3.5 log units with the nisin-phage mixtures on honeydew melon and apple, respectively, was greater than the one produced by nisin alone and even greater than the one obtained with aqueous chemical sanitizers (Leverentz et al., 2003).

Nisin and a commercial preparation of the polyvalent P100 phage (Listex™ P100) were applied to cold-smoked salmon. Small pieces of salmon were inoculated with a solution of roughly 10^6 cfu/mL of a five-strain cocktail of *L. monocytogenes* and, after 15 min, different treatments were applied. Samples were analyzed after 24 h at 4 °C. While individual treatments achieved a reduction of 2–3 log units, the phage: bacteriocin combination lowered the bacterial population to undetectable levels (Soni et al., 2014). This combination was also studied in ready-to-eat sliced pork ham, which was inoculated with approximately 10^4 cfu/mL of *L. monocytogenes* and treated using a sterile glass spreader. *L. monocytogenes* was analyzed immediately and after 3 days stored at 6–8 °C. At first, nisin and phage alone were effective in reducing the initial contamination, while the combination only had a small effect, showing antagonism between these antimicrobials. However, after 72 h

Table 1
Examples of the combined use of bacteriocins and bacteriophages or phage endolysins.

Pathogen	Bacteriocin	Phage product	Matrix (food)	Main results	Reference
L. monocytogenes	Nisin (Nisaplin)	Mixtures LM-103 and LMP-102	Apple Honeydew melon	Synergy; better than traditional sanitizers	(Leverentz et al., 2003)
	Nisin	P100 (Listex P100)	Cold-smoked salmon	Synergy; active against a five-strain cocktail.	(Soni et al., 2014)
	Nisin	P100	Pork ham	Better than nisin alone after 3 days of storage	(Figueiredo and Almeida, 2017)
	Nisin (Nisaplin)	P100	Coleslaw	Not better than phage alone; no resistance observed	(Lewis et al., 2019)
	Nisin	PlyP100 (endolysin)	Queso fresco	Strong synergy with no resistance development	(Ibarra-Sánchez et al., 2018)
	Nisin	LH7	Chilled beef	Synergy in broth but not in beef	(Dykes and Moorhead, 2002)
	Enterocin AS-48	P100	Raw hake Raw and smoked salmon	Synergy	(Baños et al., 2016)
	Coagulin C23	FWLLm1, FWLLm3	Milk	Synergy; lower frequency of resistant mutants	(Rodríguez-Rubio et al., 2015)
	Pediocin PA1	P100 (Listex P100)	Milk	Synergy	(Komora et al., 2020)
S. aureus	Nisin	phi35, phi88	Milk	Synergy; cross-resistance after prolonged incubation	(Martínez et al., 2008)
	Nisin	SA46-CTH2	Milk	Synergy in broth, but not in milk or against biofilms	(Duc et al., 2020)
	Nisin	LysH5 (endolysin)	Milk	Synergy; no cross-resistance	(García et al., 2010a)
	From *L. lactis* CJNU 3001	SAP 84	*In vitro*	Synergy	(Kim et al., 2019)
	Lysostaphin	LysK (endolysin)	*In vitro*	Synergy	(Becker et al., 2008)
	Lysostaphin	CHAPK (truncated LysK endolysin)	*In vitro*	Synergy	(Hathaway et al., 2017)
Salmonella	Nisin	Fmb-p1	Chilled pork	No synergy; improvement of food quality and safety parameters	(Wang et al., 2017)
	Nisin	P22	Biofilms	Synergy in preventing biofilm formation and biofilm removal	(Yüksel et al., 2018)
C. perfringens	From *S. hyointestinalis* B19	P4, A3	*In vitro*	Synergy	(Heo et al., 2018)

the bacteriocin-phage combination achieved a reduction of 3 log units in the number of viable cells, which was greater than the reduction with nisin and comparable to the one achieved with phage alone (Figueiredo and Almeida, 2017). Similarly, Lewis et al. studied the combination of Nisaplin and P100 both *in vitro* and in coleslaw, with contrasting results. First, two checkerboard assays were performed in TBS and coleslaw liquid and incubated for 24 h at 30 °C and 4 °C, respectively, to determine the Fractional Inhibitory Concentration (FIC). While no synergy was observed in broth, partial synergy (FIC = 0.6) between P100 at a Multiplicity of Infection (MOI) of 2.5 and Nisaplin at 25 μg/mL was observed. These conditions were selected for a food trial, where coleslaw was inoculated with 10^7 cfu/mL of *L. monocytogenes* and then treated and stored at 4 °C for 10 days. With the combined treatment, the number of cells was reduced from 9.4×10^5 to 1.2×10^2 cfu/mL by day 10, with no statistically significant difference between this combination and phage alone, but significantly better than Nisaplin alone. Moreover, after analyzing the resistance to P100 and Nisaplin of several colonies on day 10 after exposure to both antimicrobials, no colonies were resistant to P100 or Nisaplin, concluding that the possibility of developing resistance to both antimicrobials is extremely low (Lewis et al., 2019).

Related to this, another study performed in Queso Fresco combined nisin with PlyP100, the endolysin from phage P100. In this case, nisin was added directly to the milk before renneting, while PlyP100 and the *L. monocytogenes* inoculum were incorporated into drained curd before pressing. Samples were analyzed after 28 days of cold storage, showing an evident synergy. Nisin alone slowed down *L. monocytogenes* growth over the first week but was not effective in the long term. The endolysin alone resulted in a 0.5 log cfu/g reduction, unable to ensure food safety. On the contrary, the synergistic effect of both antimicrobials together reduced the viable cells below the detection limit and avoided re-growth after 48 h of enrichment in about half of the samples. As in other studies, the susceptibility to nisin or PlyP100 did not change after the exposure to these substances, suggesting that there was no resistance development (Ibarra-Sánchez et al., 2018).

Despite the synergy observed between nisin and phages in several examples, this is not always the case. Dykes and Moorhead (2002) analyzed the combination of Nisaplin and the phage LH7 in broth, chilled buffer and chill-stored vacuum packaged beef stored at different temperatures (30.7 and 4 °C) for 4 weeks. An enhanced effect of the combined use was observed in broth, especially at 7 °C and even in stationary phase cells. However, this effect was not observed in buffer or meat, where nisin alone had the most significant effect (Dykes and Moorhead, 2002). These results further exemplify the complexity of food models, in which the nature of the food matrix may hinder phage:host encounters or inactivate phages (Hagens and Loessner, 2010).

Although nisin is the most commonly used bacteriocin, it is not the only example. The efficacy of the circular enterocin AS-48 and phage P100 alone or in combination was also tested in different kinds of fishes. Raw hake, raw salmon and smoked salmon were inoculated with a suspension of 5×10^5 cfu/mL of *L. monocytogenes* and then treated using an automated spray system and stored at 4 °C for 7 days in the case of raw fish and 30 days for smoked fish. The use of AS-48 alone resulted in an important reduction of *L. monocytogenes* in raw salmon, raw hake and smoked salmon (up to 3.13, 2.8 and 4.25 log cfu/mL, respectively). Similarly, phage alone reduced up to 2.02, 1.25 and 2.75 log cfu/mL *L. monocytogenes* cell counts. However, the most drastic effect was observed when both antimicrobials were combined, when the *L. monocytogenes* population was kept under the detection limit until day 7 in raw hake, from day 2 to day 7 in raw salmon and until day 15 in smoked salmon (Baños et al., 2016).

Finally, two more studies addressed the combination of phage-bacteriocin against this pathogen, both applied in whole milk. The combination of coagulin C23, a class IIa bacteriocin and the myoviruses FWLLm1 and FWLLm3 was analyzed first under optimal growth

conditions in broth showing that the combination was more effective than each antimicrobial alone. Their effectiveness in milk contaminated with 5×10^4 cfu/mL and stored for 10 days at 4 °C was also assessed. The C23-FWLLm1 combination reduced viable cells to levels below the detection limit by day two, and no regrowth was observed after that. In contrast, the combination with phage FWLLm3 resulted in a reduction of 7.5 log units on day 4, followed by a 1.7 log units increase at the end of the experiment. Remarkably, the frequency of resistant mutants to either C23 or phage FWLLm3 isolated from samples treated with both antimicrobials was lower than that found in the single treatments, which may explain the better performance of the combined strategy (Rodríguez-Rubio et al., 2015). Komora et al. (2020) combined high hydrostatic pressure (HHP) with pediocin PA-1 and the phage P100 (Listex P100) in milk contaminated with 10^4 and 10^7 cfu/mL of *L. monocytogenes* and compared the results with those obtained with traditional thermal methods. A synergistic effect was observed between HHP, pediocin PA-1 and phage P100 at the two levels of contamination. However, while results were comparable to those obtained with high-temperature, short-time pasteurization at the low contamination level, this was not the case when contamination was high, where the thermal treatment was more effective (Komora et al., 2020).

4.2. Staphylococcus aureus

S. aureus is a common target in food biopreservation because it is frequently involved in food poisoning and a frequent cause of bovine mastitis, which contributes to its ubiquitous presence in milk. Moreover, resistance to antibiotics is often encountered in nosocomial infections (Hennekinne et al., 2012; Munita et al., 2015).

To the best of our knowledge, there are three studies about the combination of nisin and phages or phage-based products in milk against this bacterium. The first trial was reported by Martínez et al. (2008), combining nisin with the lytic phages phi35 and phi88 in commercial pasteurized milk inoculated with 10^7 cfu/mL of *S. aureus* Sa9 and incubated at 37 °C. Although a synergistic effect was observed when the antimicrobials were used at sub-optimal concentrations, bacteriophage activity after prolonged incubations was compromised. This could be linked to adaptive changes triggered by nisin that modified the surface of *S. aureus* cells, weakening phage infectivity (Martínez et al., 2008). Interestingly, bacteriophage insensitive mutants did not show cross-resistance to nisin.

Duc et al. (2020) tested the combination of nisin and phage SA46-CTH2, a *Podoviridae* phage isolated from a food sample. The authors determined the efficacy of this combination for reducing *S. aureus* contamination in broth and milk, as well as for eliminating biofilms on polystyrene and stainless steel. The combination of nisin and phage resulted in the inhibition of *S. aureus* regrowth in broth, with a higher efficacy than either treatment alone. However, such synergistic effect was not observed in milk and on biofilm cells (Duc et al., 2020).

Remarkably, the lytic activity of some phage endolysins is enhanced in the presence of ionophores that disrupt the bacterial membrane proton motive force (Escobedo et al., 2019; Nascimento et al., 2008). In this line, LysH5, the endolysin from the *Staphylococcus* phage phi88, was tested in combination with nisin in commercial pasteurized milk inoculated with 10^2 and 10^5 cfu/mL of *S. aureus* Sa9 (García et al., 2010a). The synergistic effect observed in broth was also confirmed in milk. While LysH5 and nisin alone had a small inhibitory effect, their combination kept the number of cells below the detection limit after 6 h of incubation, being the reduction noticeable after 4 h. In this case, nisin resistant mutants were still sensitive to the endolysin, which constitutes an advantage compared to the use of phages as described above (Martínez et al., 2008).

Other studies dealing with the combination of phages or phage endolysins and bacteriocins against *S. aureus* can be found, although their efficacy in food models has not been tested. Kim et al. combined an unidentified bacteriocin from *L. lactis* CJNU 3001 and the *Staphylococcus* phage SAP84. While the bacteriocin at 100 AU/mL reduced the viable cells by 1.3 log cfu/mL and the phage alone at MOI 0.1 was unable to inhibit growth, the combination of the phage at the same MOI and the bacteriocin at 50 and 100 AU/mL achieved a reduction of 0.9 and 2.5 log cfu/mL, respectively, which suggests a synergistic effect (Kim et al., 2019).

Regarding phage endolysins, two trials reported their pairing with lysostaphin, a powerful anti-staphylococcal lytic bacteriocin, showing synergy in checkerboard tests in both cases. Combination of lysostaphin with LysK, the endolysin of phage K, showed a greater inhibition than each protein alone, with a FIC of 0.45 (Becker et al., 2008). When combined with the endolysin $CHAP_K$, a truncated version of LysK, a strong synergy was also observed at several concentrations, with FICs ranging from 0.144 to 0.378 (Hathaway et al., 2017).

4.3. Salmonella spp.

Salmonella is the second most reported zoonosis in humans (European Food Safety Authority and European Centre for Disease Prevention and Control, 2021). As a Gram-negative bacterium, its outer membrane protects cells against the activity of pore-forming bacteriocins, unless high concentrations or outer membrane disrupting agents such as organic acids or EDTA are used (Gálvez et al., 2010). Two studies reported on the combination of nisin with phages. The efficacy of the *Salmonella* phage fmb-p1, together with nisin and potassium sorbate, was tested in fresh chilled pork against *Salmonella Typhimurium*, as well as its effect on several organoleptic properties (Wang et al., 2017). Small pieces of meat were inoculated with 10^3 cfu/mL, treated and stored at 4 °C for 21 days. All the phage treatments, alone or in all kinds of combinations, were able to reduce *Salmonella* counts below the detection limit, while nisin and potassium sorbate individually or in combination failed. Although no synergy between the antimicrobials was observed, the triple combination was the best option to improve some parameters such as the TVC (Total Viable Count), the pH or the spoilage time, extending the shelf life of this product to up to 14 days at 4 °C (Wang et al., 2017). Additionally, the use of the phage P22 and nisin-EDTA was assessed in order to prevent *Salmonella Typhimurium* biofilm formation and to disrupt mature biofilms (Yüksel et al., 2018). The results of this exhaustive study showed that over 93% inhibition of biofilm formation could be achieved with the combined treatment. Interestingly, when used in combination, the titer of phage P22 to reach maximum reduction values (10^2 pfu/mL) was, at least, three orders of magnitude lower than when the phage was used alone. Eliminating mature biofilm proved to be more difficult, but up to 70% was accomplished with the triple combination containing 10^7 pfu/mL P22, 20 mM EDTA and 150 μg/mL of nisin (Yüksel et al., 2018).

4.4. Clostridium perfringens

C. perfringens produces several toxins that cause diarrhea and can be found in raw meat and poultry. While the disease is not often reported and people recover without treatment, *C. perfringens* is a main causative agent of intestinal disorders in farm animals that require antibiotic treatment (Kiu and Hall, 2018).

Combination of a bacteriocin produced by *Streptococcus hyointestinalis* B19 with two phages isolated from chicken and pig feces, as well as the combination of the bacteriocin-producing strain with the phages was studied in broth. A synergistic effect was observed between bacteriocin and phages, reaching a reduction of 6.20 log units of the bacterial population, while the efficacy of each antimicrobial alone was 3.8 log units for the bacteriocin and 1.36 and 4.41 log units for phages P4 and A3, respectively (Heo et al., 2018). The authors also checked inhibition of *C. perfringens* in co-culture with the bacteriocin producer plus the two *C. perfringens* phages. In this case, complete eradication of *C. perfringens* was achieved (Heo et al., 2018).

4.5. Looking for novel biotech solutions

Besides the "simple" recipe of using bacteriocins, phages and endolysins in combination to control bacterial growth, several biotechnological alternatives are also being developed exploiting the existing knowledge on their biology.

One of the examples is the heterologous production of phage endolysins by the dairy starter *L. lactis* making use of bacteriocin-based gene expression tools such as the nisin-inducible (NICE) system based on the nisin autoinducing properties (Kuipers et al., 1995). Indeed, the first developed application of the NICE system was to express the lytic genes of the lactococcal phage phiUS3 with the aim of triggering lysis of lactococcal cells in order to accelerate cheese maturation by enabling the release of intracellular enzymes (De Ruyter et al., 1997). The NICE system was also used for the heterologous production of the anti-staphylococcal endolysin LysH5 in *L. lactis* (Rodríguez-Rubio et al., 2012a). In this example, the Lcn972 bacteriocin signal peptide was fused to drive secretion of the active endolysin. However, although secretion was achieved after the addition of nisin, very low lytic activity was detected in the supernatants from co-cultures of the lactococcal clones with a nisin-producing *L. lactis*. More recently, the efficacy of a nisin-producing *L. lactis* expressing engineered versions of the endolysin CTP1L gene under the control of the nisin promoter has been assessed to fight against *C. tyrobutyricum*, a food-spoiling bacterium responsible for the late blowing defect in cheese, which causes texture and flavor defects (Garde et al., 2020). While the presence of the different endolysin-expressing plasmids allowed the constitutive expression and secretion of the endolysin without affecting most of the technological properties of *L. lactis*, nisin production was very low (1.95–3 IU/mL), compared with the wildtype strain (>300 IU/mL). Both the nisin-producing L. *lactis* and the endolysin-producing clones were able to delay the late blowing defect by one month, being the endolysin more effective than nisin on reducing *C. tyrobutyricum* counts (Garde et al., 2020).

Another approach reported in the literature is the creation of chimeric antimicrobial proteins, joining domains from bacteriocins and endolysins which often results in enhanced activity or specificity. For example, fusions of a virion-associated peptidoglycan hydrolase HydH5 or the CHAP catalytic domain of the LysK endolysin with the cell wall binding domain of lysostaphin showed higher staphylolytic activity than their parental proteins (Arroyo-Moreno et al., 2021; Rodríguez-Rubio et al., 2012b).

A similar strategy has been applied to help phage endolysins to overcome the protecting role of the outer membrane in Gram-negative bacteria. The first engineered endolysin targeting Gram-negative bacteria was created by fusion of the N-terminal FyuA-targeting domain of pesticin, a class III cell wall targeting bacteriocin, to the phage T4 lysozyme (Lukacik et al., 2012). This pesticin-lysozyme hybrid killed bacteria expressing *fyuA*, a virulence factor of several human pathogens such as *Yersinia pestis* and *E. coli*, including the O104:H4 strain (Lukacik et al., 2012).

The modification of class III multi-complex bacteriocins with phage tail proteins has also been described. A *Pseudomonas aeruginosa* R-type pyocin was retargeted towards *E. coli* O157:H7 by fusing an O157-specific tail spike protein from a *Podoviridae* phage (Scholl et al., 2009). This modified pyocin is highly specific and able to reduce *E. coli* Shiga-toxin producers from beef surfaces below the detection level. Likewise, Heselpoth et al. (2019) combined the PyS2 domains I to III of a S-type pyocin and the lysin GN4 from a *P. aeruginosa* phage, being this so-called lysocin effective on planktonic and sessile *P. aeruginosa* cells (Heselpoth et al., 2019). These are just a couple of examples to showcase the potential of swapping the target recognition domains of class III multi-complex bacteriocins by phage receptor binding proteins to produce antimicrobials with a customized spectrum as reviewed elsewhere (Dams et al., 2019). Overall, these strategies show the multiple opportunities of developing new-to-nature antimicrobial proteins to efficiently target Gram-negative bacteria that represent a serious burden in food safety.

There is yet another biotech solution which has been recently described. The virulent *E. coli* T7 phage has been engineered to encode the leaderless-bacteriocin lacticin Q that exerts strong antibacterial activity against Gram-positive bacteria, including *Bacillus cereus* and *S. aureus*, among others (Masuda et al., 2021). In this proof-of-concept study, it is shown that this modified phage is able to kill *Bacillus coagulans* while infecting its *E. coli* host. Interestingly, bacteriocin-producing phages are not "unnatural" and, although rare, they have been detected in nature, predominately in temperate phages, and may confer a competitive advantage to lysogens in complex ecosystems (Dragoš et al., 2021).

5. Future directions and recommendations

The efforts invested in developing food biopreservation strategies based on either bacteriocins or bacteriophages have unveiled the advantages and challenges of each antimicrobial concerning their effectiveness in complex food matrices. To overcome these caveats, scientists have just started to explore their efficacy when applied together as a new means to curtail the presence of undesirable bacteria in food. Specific research needs and future directions of this strategy have been identified as outlined in Table 2. Notably, most of the available data comes from *in vitro* assays and only a few reports have addressed their efficacy in food models. Moreover, the number of target species is still limited and bacterial targets other than pathogenic bacteria have been hardly addressed. Likewise, nisin has been so far the bacteriocin of choice in most studies and information about other bacteriocins with different modes of action is still lacking.

Overall, the available body of knowledge on this topic is still insufficient to rationalize this multi-hurdle approach. In fact, finding common trends is a major bottleneck due to the immense structural and functional diversity within both bacteriocins and bacteriophages. Nevertheless, as new experimental data comes to light, along with a deeper understanding of the mechanisms behind synergy or antagonism, it will be possible to provide specific guidelines to foster the implementation of this approach and its acceptance by consumers, food stakeholders and the competent authorities.

6. Conclusion

Aware that a "silver bullet" to reconcile consumer demands for natural products and the strict measures necessary to ensure food safety is far from being on hand, the results reported so far about the efficacy of the combined used of bacteriocins and bacteriophages are encouraging because synergistic (or additive) effects are often observed. Adopting such a multi-hurdle approach will help us move towards a more sustainable food production chain.

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Table 2
Research needs and future directions regarding the implementation of a multi-hurdle approach based on the combination of bacteriocins and bacteriophages.

Research needs	Future directions
Interactions with the food matrix	Further testing in food models
Impact on shelf-life	Expand the bacterial targets to include spoiling bacteria
Systematic approach to find common trends	Studies using bacteriocins with different modes of action and phage cocktails
Understanding the observed effects	Mechanistic insight into bacteriocin:phage interactions

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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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References

Acedo, J.Z., Chiorean, S., Vederas, J.C., van Belkum, M.J., 2018. The expanding structural variety among bacteriocins from Gram-positive bacteria. FEMS Microbiol. Rev. 42, 805–828. https://doi.org/10.1093/femsre/fuy033.

Alvarez-Sieiro, P., Montalbán-López, M., Mu, D., Kuipers, O.P., 2016. Bacteriocins of lactic acid bacteria: extending the family. Appl. Microbiol. Biotechnol. 100, 2939–2951. https://doi.org/10.1007/s00253-016-7343-9.

Arroyo-Moreno, S., Begley, M., Dembicka, K., Coffey, A., 2021. Engineering of the chapk staphylococcal phage endolysin to enhance antibacterial activity against stationary-phase cells. Antibiotics 10, 722. https://doi.org/10.3390/antibiotics10060722.

Asioli, D., Aschemann-Witzel, J., Caputo, V., Vecchio, R., Annunziata, A., Næs, T., Varela, P., 2017. Making sense of the "clean label" trends: a review of consumer food choice behavior and discussion of industry implications. Food Res. Int. 99, 58–71. https://doi.org/10.1016/j.foodres.2017.07.022.

Baños, A., García-López, J.D., Núñez, C., Martínez-Bueno, M., Maqueda, M., Valdivia, E., 2016. Biocontrol of *Listeria monocytogenes* in fish by enterocin AS-48 and *Listeria* lytic bacteriophage P100. LWT Food Sci. Technol. 66, 672–677. https://doi.org/10.1016/j.lwt.2015.11.025.

Bastos Mdo, C., Coelho, M.L., Santos, O.C., 2015. Resistance to bacteriocins produced by Gram-positive bacteria. Microbiol−SGM 161, 683–700. https://doi.org/10.1099/mic.0.082289-0.

Becker, S.C., Foster-Frey, J., Donovan, D.M., 2008. The phage K lytic enzyme LysK and lysostaphin act synergistically to kill MRSA. FEMS Microbiol. Lett. 287, 185–191. https://doi.org/10.1111/j.1574-6968.2008.01308.x.

Benítez-Chao, D.F., León-Buitimea, A., Lerma-Escalera, J.A., Morones-Ramírez, J.R., 2021. Bacteriocins: an overview of antimicrobial, toxicity, and biosafety assessment by *in vivo* models. Front. Microbiol. 12, 677. https://doi.org/10.3389/fmicb.2021.630695.

Briers, Y., Walmagh, M., Van Puyenbroeck, V., Cornelissen, A., Cenens, W., Aertsen, A., Oliveira, H., Azeredo, J., Verween, G., Pirnay, J.P., Miller, S., Volckaert, G., Lavigne, R., 2014. Engineered endolysin-based "Artilysins" to combat multidrug-resistant gram-negative pathogens. MBio 5. https://doi.org/10.1128/MBIO.01379-14 e01379-e01314.

Brötz, H., Josten, M., Wiedemann, I., Schneider, U., Götz, F., Bierbaum, G., Sahl, H.G., 1998. Role of lipid-bound peptidoglycan precursors in the formation of pores by nisin, epidermin and other lantibiotics. Mol. Microbiol. 30, 317–327. https://doi.org/10.1046/j.1365-2958.1998.01065.x.

Campelo, A.B., López-González, M.J., Escobedo, S., Janzen, T., Neves, A.R., Rodríguez, A., Martínez, B., 2020. Mutations selected after exposure to bacteriocin Lcn972 activate a Bce-like bacitracin resistance module in *Lactococcus lactis*. Front. Microbiol. 11, 1805. https://doi.org/10.3389/fmicb.2020.01805.

Cebrián, R., Martínez-Bueno, M., Valdivia, E., Albert, A., Maqueda, M., Sánchez-Barrena, M.J., 2015. The bacteriocin AS-48 requires dimer dissociation followed by hydrophobic interactions with the membrane for antibacterial activity. J. Struct. Biol. 190, 162–172. https://doi.org/10.1016/j.jsb.2015.03.006.

Chan, B.K., Abedon, S.T., Loc-Carrillo, C., 2013. Phage cocktails and the future of phage therapy. Future Microbiol. 8, 769–783. https://doi.org/10.2217/FMB.13.47.

Cotter, P.D., Hill, C., Ross, R.P., 2005. Bacteriocins: developing innate immunity for food. Nat. Rev. Microbiol. 3, 777–788. https://doi.org/10.1038/nrmicro1273.

Dams, D., Brøndsted, L., Drulis-Kawa, Z., Briers, Y., 2019. Engineering of receptor-binding proteins in bacteriophages and phage tail-like bacteriocins. Biochem. Soc. Trans. 47, 449–460. https://doi.org/10.1042/BST20180172.

Daniel, A., Euler, C., Collin, M., Chahales, P., Gorelick, K.J., Fischetti, V.A., 2010. Synergism between a novel chimeric lysin and oxacillin protects against infection by methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. 54, 1603–1612. https://doi.org/10.1128/AAC.01625-09.

De Ruyter, P.G.G.A., Kuipers, O.P., Meijer, W.C., De Vos, W.M., 1997. Food-grade controlled lysis of *Lactococcus lactis* for accelerated cheese ripening. Nat. Biotechnol. 15, 976–979. https://doi.org/10.1038/NBT1097-976.

D'Hérelle, F., Smith, G.H., 1930. In: Charles, C. (Ed.), The Bacteriophage and Its Clinical Application. Thomas, Springfield, IL, USA.

Diep, D.B., Skaugen, M., Salehian, Z., Holo, H., Nes, I.F., 2007. Common mechanisms of target cell recognition and immunity for class II bacteriocins. Proc. Natl. Acad. Sci. U. S. A. 104, 2384–2389, 0608775104 [pii]10.1073/pnas.0608775104.

Dragoš, A., Andersen, A.J.C., Lozano-Andrade, C.N., Kempen, P.J., Kovács, Á.T., Strube, M.L., 2021. Phages carry interbacterial weapons encoded by biosynthetic gene clusters. Curr. Biol. 31, 3479–3489.e5. https://doi.org/10.1016/J.CUB.2021.05.046.

Draper, L.A., Cotter, P.D., Hill, C., Ross, R.P., 2015. Lantibiotic resistance. Microbiol. Mol. Biol. Rev. 79, 171–191. https://doi.org/10.1128/MMBR.00051-14.

Duarte, A.C., Fernández, L., De Maesschalck, V., Gutiérrez, D., Campelo, A.B., Briers, Y., Lavigne, R., Rodríguez, A., García, P., 2021. Synergistic action of phage phiIPLA-RODI and lytic protein CHAPSH3b: a combination strategy to target *Staphylococcus aureus* biofilms. npj Biofilms Microbiomes 7, 39. https://doi.org/10.1038/S41522-021-00208-5.

Duc, H.M., Son, H.M., Ngan, P.H., Sato, J., Masuda, Y., Honjoh, K.ichi, Miyamoto, T., 2020. Isolation and application of bacteriophages alone or in combination with nisin against planktonic and biofilm cells of *Staphylococcus aureus*. Appl. Microbiol. Biotechnol. 104, 5145–5158. https://doi.org/10.1007/s00253-020-10581-4.

Duraisamy, S., Balakrishnan, S., Ranjith, S., Husain, F., Sathyan, A., Ansu, Peter, S., Prahalathan, C., Kumarasamy, A., 2020. Bacteriocin-a potential antimicrobial peptide towards disrupting and preventing biofilm formation in the clinical and environmental locales. Environ. Sci. Pollut. Res. 27, 44922–44936. https://doi.org/10.1007/s11356-020-10989-5.

Dykes, G.A., Moorhead, S.M., 2002. Combined antimicrobial effect of nisin and a listeriophage against *Listeria monocytogenes* in broth but not in buffer or on raw beef. Int. J. Food Microbiol. 73, 71–81. https://doi.org/10.1016/S0168-1605(01)00710-3.

Ennahar, S., Deschamps, N., Richard, J., 2000. Natural variation in susceptibility of Listeria strains to class IIa bacteriocins. Curr. Microbiol. 41, 1–4. https://doi.org/10.1007/s002840010081.

Escobedo, S., Campelo, A.B., Wegmann, U., García, P., Rodríguez, A., Martínez, B., 2019. Insight into the lytic functions of the lactococcal prophage TP712. Viruses 11, 881. https://doi.org/10.3390/V11100881.

European Food Safety Authority and European Centre for Disease Prevention and Control, 2021. The European Union One Health 2019 Zoonoses Report. EFSA J. 19, e06406 https://doi.org/10.2903/j.efsa.2021.6406.

Fernández, L., Gutiérrez, D., Rodríguez, A., García, P., 2018. Application of bacteriophages in the agro-food sector: a long way toward approval. Front. Cell. Infect. Microbiol. 8, 296. https://doi.org/10.3389/FCIMB.2018.00296.

Field, D., Cotter, P.D., Hill, C., Ross, R.P., 2015. Bioengineering lantibiotics for therapeutic success. Front. Microbiol. 6, 1363. https://doi.org/10.3389/fmicb.2015.01363.

Figueiredo, A.C.L., Almeida, R.C.C., 2017. Antibacterial efficacy of nisin, bacteriophage P100 and sodium lactate against *Listeria monocytogenes* in ready-to-eat sliced pork ham. Braz. J. Microbiol. 48, 724–729. https://doi.org/10.1016/j.bjm.2017.02.010.

Gálvez, A., Abriouel, H., Benomar, N., Lucas, R., 2010. Microbial antagonists to food-borne pathogens and biocontrol. Curr. Opin. Biotechnol. 21, 142–148. https://doi.org/10.1016/j.copbio.2010.01.005.

García, P., Martínez, B., Rodríguez, L., Rodríguez, A., 2010a. Synergy between the phage endolysin LysH5 and nisin to kill *Staphylococcus aureus* in pasteurized milk. Int. J. Food Microbiol. 141, 151–155. https://doi.org/10.1016/j.ijfoodmicro.2010.04.029.

García, P., Rodríguez, L., Rodríguez, A., Martínez, B., 2010b. Food biopreservation: promising strategies using bacteriocins, bacteriophages and endolysins. Trends Food Sci. Technol. 21, 373–382. https://doi.org/10.1016/j.tifs.2010.04.010.

Garcia-Gutierrez, E., Mayer, M.J., Cotter, P.D., Narbad, A., 2019. Gut microbiota as a source of novel antimicrobials. Gut Microbes 10, 1–21. https://doi.org/10.1080/19490976.2018.1455790.

Garde, S., Calzada, J., Sánchez, C., Gaya, P., Narbad, A., Meijers, R., Mayer, M.J., Ávila, M., 2020. Effect of *Lactococcus lactis* expressing phage endolysin on the late blowing defect of cheese caused by *Clostridium tyrobutyricum*. Int. J. Food Microbiol. 329, 108686 https://doi.org/10.1016/j.ijfoodmicro.2020.108686.

Gondil, V.S., Harjai, K., Chhibber, S., 2020. Endolysins as emerging alternative therapeutic agents to counter drug-resistant infections. Int. J. Antimicrob. Agents 55, 105844. https://doi.org/10.1016/J.IJANTIMICAG.2019.11.001.

Gutiérrez, D., Rodríguez-Rubio, L., Martínez, B., Rodríguez, A., García, P., 2016. Bacteriophages as weapons against bacterial biofilms in the food industry. Front. Microbiol. 7, 825. https://doi.org/10.3389/fmicb.2016.00825.

Gutiérrez, D., Fernández, L., Rodríguez, A., García, P., 2018. Are phage lytic proteins the secret weapon to kill *Staphylococcus aureus*? MBio 9. https://doi.org/10.1128/MBIO.01923-17 e01923-e01917.

Gutiérrez, D., Garrido, V., Fernández, L., Portilla, S., Rodríguez, A., Grilló, M.J., García, P., 2020. Phage lytic protein LysRODI prevents staphylococcal mastitis in mice. Front. Microbiol. 11, 7. https://doi.org/10.3389/fmicb.2020.00007.

Hagens, S., Loessner, M., 2010. Bacteriophage for biocontrol of foodborne pathogens: calculations and considerations. Curr. Pharm. Biotechnol. 11, 58–68. https://doi.org/10.2174/138920110790725429.

Hathaway, H., Ajuebor, J., Stephens, L., Coffey, A., Potter, U., Sutton, J.M., Jenkins, A.T. A., 2017. Thermally triggered release of the bacteriophage endolysin CHAPK and the bacteriocin lysostaphin for the control of methicillin resistant *Staphylococcus aureus* (MRSA). J. Control. Release 245, 108–115. https://doi.org/10.1016/j.jconrel.2016.11.030.

Hennekinne, J.A., De Buyser, M.L., Dragacci, S., 2012. *Staphylococcus aureus* and its food poisoning toxins: characterization and outbreak investigation. FEMS Microbiol. Rev. 36, 815–836. https://doi.org/10.1111/J.1574-6976.2011.00311.X.

Heo, S., Kim, M.G., Kwon, M., Lee, H.S., Kim, G.B., 2018. Inhibition of *Clostridium perfringens* using bacteriophages and bacteriocin producing strains. Korean J. Food Sci. Anim. Resour. 38, 88–98. https://doi.org/10.5851/kosfa.2018.38.1.88.

Heselpoth, R.D., Euler, C.W., Schuch, R., Fischetti, V.A., 2019. Lysocins: bioengineered antimicrobials that deliver lysins across the outer membrane of Gram-negative bacteria. Antimicrob. Agents Chemother. 63, 1–14. https://doi.org/10.1128/AAC.00342-19.

Ibarra-Sánchez, L.A., Van Tassell, M.L., Miller, M.J., 2018. Antimicrobial behavior of phage endolysin PlyP100 and its synergy with nisin to control *Listeria monocytogenes* in Queso Fresco. Food Microbiol. 72, 128–134. https://doi.org/10.1016/j.fm.2017.11.013.

Ibarra-Sánchez, L.A., El-Haddad, N., Mahmoud, D., Miller, M.J., Karam, L., 2020. Invited review: advances in nisin use for preservation of dairy products. J. Dairy Sci. 103, 2041–2052. https://doi.org/10.3168/jds.2019-17498.

Johnson, E.M., Jung, D.Y.G., Jin, D.Y.Y., Jayabalan, D.R., Yang, D.S.H., Suh, J.W., 2018. Bacteriocins as food preservatives: challenges and emerging horizons. Crit. Rev. Food Sci. Nutr. 58, 2743–2767. https://doi.org/10.1080/10408398.2017.1340870.

Keen, E.C., Bliskovsky, V.V., Malagon, F., Baker, J.D., Prince, J.S., Klaus, J.S., Adhya, S. L., 2017. Novel "superspreader" bacteriophages promote horizontal gene transfer by transformation. MBio 8, e02115–e02116. https://doi.org/10.1128/MBIO.02115-16.

Kim, S.G., Lee, Y.D., Park, J.H., Moon, G.S., 2019. Synergistic inhibition by bacteriocin and bacteriophage against *Staphylococcus aureus*. Food Sci. Anim. Resour. 39, 1015–1020. https://doi.org/10.5851/kosfa.2019.e95.

Kiu, R., Hall, L.J., 2018. An update on the human and animal enteric pathogen *Clostridium perfringens*. Emerg. Microbes Infect. 7, 1–15. https://doi.org/10.1038/S41426-018-0144-8.

Kjos, M., Oppegård, C., Diep, D.B., Nes, I.F., Veening, J.W., Nissen-Meyer, J., Kristensen, T., 2014. Sensitivity to the two-peptide bacteriocin lactococcin G is dependent on UppP, an enzyme involved in cell-wall synthesis. Mol. Microbiol. 92, 1177–1187. https://doi.org/10.1111/mmi.12632.

Komora, N., Maciel, C., Pinto, C.A., Ferreira, V., Brandão, T.R.S., Saraiva, J.M.A., Castro, S.M., Teixeira, P., 2020. Non-thermal approach to *Listeria monocytogenes* inactivation in milk: the combined effect of high pressure, pediocin PA-1 and bacteriophage P100. Food Microbiol. 86, 103315 https://doi.org/10.1016/j.fm.2019.103315.

Kuipers, O.P., Beerthuyzen, M.M., De Ruyter, P.G.G.A., Luesink, E.J., De Vos, W.M., 1995. Autoregulation of nisin biosynthesis in *Lactococcus lactis* by signal transduction. J. Biol. Chem. 270, 27299–27304. https://doi.org/10.1074/JBC.270.45.27299.

Leistner, L., 1992. Food preservation by combined methods. Food Res. Int. 25, 151–158. https://doi.org/10.1016/0963-9969(92)90158-2.

Leverentz, B., Conway, W.S., Camp, M.J., Janisiewicz, W.J., Abuladze, T., Yang, M., Saftner, R., Sulakvelidze, A., 2003. Biocontrol of *Listeria monocytogenes* on fresh-cut produce by treatment with lytic bacteriophages and a bacteriocin. Appl. Environ. Microbiol. 69, 4519–4526. https://doi.org/10.1128/AEM.69.8.4519-4526.2003.

Lewis, R., Hill, C., 2020. Overcoming barriers to phage application in food and feed. Curr. Opin. Biotechnol. 61, 38–44. https://doi.org/10.1016/J.COPBIO.2019.09.018.

Lewis, R., Bolocan, A.S., Draper, L.A., Paul Ross, R., Hill, C., 2019. The effect of a commercially available bacteriophage and bacteriocin on *Listeria monocytogenes* in coleslaw. Viruses 11, 977. https://doi.org/10.3390/v11110977.

López-Cuellar, M.D.R., Rodríguez Hernández, A.-I., Chavarría Hernández, N., 2016. LAB bacteriocin applications in the last decade. Biotechnol. Biotechnol. Equip. 30, 1–12. https://doi.org/10.1080/13102818.2016.1232605.

Lukacik, P., Barnard, T.J., Keller, P.W., Chaturvedi, K.S., Seddiki, N., Fairman, J.W., Noinaj, N., Kirby, T.L., Henderson, J.P., Steven, B.A.C., Hinnebusch, J., Buchanan, S. K., 2012. Structural engineering of a phage lysin that targets Gram-negative pathogens. Proc. Natl. Acad. Sci. U.S.A. 109, 9857–9862. https://doi.org/10.1073/pnas.1203472109.

Martínez, B., Obeso, J.M., Rodríguez, A., García, P., 2008. Nisin-bacteriophage crossresistance in *Staphylococcus aureus*. Int. J. Food Microbiol. 122, 253–258. https://doi.org/10.1016/j.ijfoodmicro.2008.01.011.

Martínez, B., García, P., Rodríguez, A., 2019. Swapping the roles of bacteriocins and bacteriophages in food biotechnology. Curr. Opin. Biotechnol. 56, 1–6. https://doi.org/10.1016/J.COPBIO.2018.07.007.

Martínez, B., Rodríguez, A., Kulakauskas, S., Chapot-Chartier, M.-P., 2020. Cell wall homeostasis in lactic acid bacteria: threats and defences. FEMS Microbiol. Rev. 44, 538–564. https://doi.org/10.1093/femsre/fuaa021.

Masuda, Y., Kawabata, S., Uedoi, T., Honjoh, K., Miyamoto, T., 2021. Construction of leaderless-bacteriocin-producing bacteriophage targeting *E. coli* and neighboring gram-positive pathogens. Microbiol. Spectr. 9 https://doi.org/10.1128/SPECTRUM.00141-21.

Miljkovic, M., Uzelac, G., Mirkovic, N., Devescovi, G., Diep, D.B., Venturi, V., Kojic, M., 2016. LsbB bacteriocin interacts with the third transmembrane domain of the YvjB receptor. Appl. Env. Microbiol. 82, 5364–5374. https://doi.org/10.1128/AEM.01293-16.

Mills, S., Ross, R.P., Hill, C., 2017. Bacteriocins and bacteriophage; a narrow-minded approach to food and gut microbiology. FEMS Microbiol. Rev. 41, S129–S153. https://doi.org/10.1093/femsre/fux022.

Montalbán-López, M., Scott, T.A., Ramesh, S., Rahman, I.R., van Heel, A.J., Viel, J.H., Bandarian, V., Dittmann, E., Genilloud, O., Goto, Y., Grande Burgos, M.J., Hill, C., Kim, S., Koehnke, J., Latham, J.A., Link, A.J., Martínez, B., Nair, S.K., Nicolet, Y., Rebuffat, S., Sahl, H.-G., Sareen, D., Schmidt, E.W., Schmitt, L., Severinov, K., Süssmuth, R.D., Truman, A.W., Wang, H., Weng, J.-K., van Wezel, G.P., Zhang, Q., Zhong, J., Piel, J., Mitchell, D.A., Kuipers, O.P., van der Donk, W.A., 2021. New developments in RiPP discovery, enzymology and engineering. Nat. Prod. Rep. 38, 130–239. https://doi.org/10.1039/D0NP00027B.

Munita, J.M., Bayer, A.S., Arias, C.A., 2015. Evolving resistance among gram-positive pathogens. Clin. Infect. Dis. 61 (Suppl. 2), S48–S57. https://doi.org/10.1093/CID/CIV523.

Murray, E., Draper, L.A., Ross, R.P., Hill, C., 2021. The advantages and challenges of using endolysins in a clinical setting. Viruses 13, 680. https://doi.org/10.3390/V13040680.

Nascimento, J.G., Guerreiro-Pereira, M.C., Costa, S.F., São-José, C., Santos, M.A., 2008. Nisin-triggered activity of Lys44, the secreted endolysin from *Oenococcus oeni* phage fOg44. J. Bacteriol. 190, 457–461. https://doi.org/10.1128/JB.01195-07.

Nelson, D., Loomis, L., Fischetti, V.A., 2001. Prevention and elimination of upper respiratory colonization of mice by group A streptococci by using a bacteriophage lytic enzyme. Proc. Natl. Acad. Sci. U. S. A. 98, 4107–4112. https://doi.org/10.1073/PNAS.061038398.

O'Sullivan, L., Bolton, D., McAuliffe, O., Coffey, A., 2019. Bacteriophages in food applications: from foe to friend. Annu. Rev. Food Sci. Technol. 10, 151–172. https://doi.org/10.1146/ANNUREV-FOOD-032818-121747.

Ricci, A., Allende, A., Bolton, D., Chemaly, M., Davies, R., Girones, R., Herman, L., Koutsoumanis, K., Lindqvist, R., Nørrung, B., Robertson, L., Ru, G., Sanaa, M., Simmons, M., Skandamis, P., Snary, E., Speybroeck, N., Ter Kuile, B., Threlfall, J., Wahlström, H., Cocconcelli, P.S., Klein, G., Prieto Maradona, M., Querol, A., Peixe, L., Suarez, J.E., Sundh, I., Vlak, J.M., Aguilera-Gómez, M., Barizzone, F., Brozzi, R., Correia, S., Heng, L., Istace, F., Lythgo, C., Fernández Escámez, P.S., 2017. Scientific opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA†. EFSA J. 15, 4664. https://doi.org/10.2903/J.EFSA.2017.4664.

Roces, C., Rodriguez, A., Martinez, B., 2012. Cell wall-active bacteriocins and their applications beyond antibiotic activity. Probiotics Antimicrob. 4, 259–272. https://doi.org/10.1007/s12602-012-9116-9.

Rodríguez-Rubio, L., Gutiérrez, D., Martínez, B., Rodríguez, A., García, P., 2012a. Lytic activity of LysH5 endolysin secreted by *Lactococcus lactis* using the secretion signal sequence of bacteriocin Lcn972. Appl. Environ. Microbiol. 78, 3469–3472. https://doi.org/10.1128/AEM.00018-12.

Rodríguez-Rubio, L., Martínez, B., Rodríguez, A., Donovan, D.M., García, P., 2012b. Enhanced staphylolytic activity of the *Staphylococcus aureus* bacteriophage vB_SauS-phiIPLA88 HydH5 virion-associated peptidoglycan hydrolase: fusions, deletions, and synergy with LysH5. Appl. Environ. Microbiol. 78, 2241–2248. https://doi.org/10.1128/AEM.07621-11.

Rodríguez-Rubio, L., García, P., Rodríguez, A., Billington, C., Hudson, J.A., Martínez, B., 2015. Listeriaphages and coagulin C23 act synergistically to kill *Listeria monocytogenes* in milk under refrigeration conditions. Int. J. Food Microbiol. 205, 68–72. https://doi.org/10.1016/j.ijfoodmicro.2015.04.007.

Schmelcher, M., Donovan, D.M., Loessner, M.J., 2012. Bacteriophage endolysins as novel antimicrobials. Future Microbiol. 7, 1147–1171. https://doi.org/10.2217/FMB.12.97.

Scholl, D., Cooley, M., Williams, S.R., Gebhart, D., Martin, D., Bates, A., Mandrell, R., 2009. An engineered R-type pyocin is a highly specific and sensitive bactericidal agent for the food-borne pathogen *Escherichia coli* O157:H7. Antimicrob. Agents Chemother. 53, 3074–3080. https://doi.org/10.1128/AAC.01660-08.

Soni, K.A., Shen, Q., Nannapaneni, R., 2014. Reduction of *Listeria monocytogenes* in cold-smoked salmon by bacteriophage P100, nisin and lauric arginate, singly or in combinations. Int. J. Food Sci. Technol. 49, 1918–1924. https://doi.org/10.1111/ijfs.12581.

Telhig, S., Ben Said, L., Zirah, S., Fliss, I., Rebuffat, S., 2020. Bacteriocins to thwart bacterial resistance in Gram negative bacteria. Front. Microbiol. 11, 2807. https://doi.org/10.3389/fmicb.2020.586433.

Tracanna, V., de Jong, A., Medema, M.H., Kuipers, O.P., 2017. Mining prokaryotes for antimicrobial compounds: from diversity to function. FEMS Microbiol. Rev. 41, 417–429. https://doi.org/10.1093/femsre/fux014.

Trejo-González, L., Gutiérrez-Carrillo, A.-E., Rodríguez-Hernández, A.-I., del Rocío López-Cuellar, M., Chavarría-Hernández, N., 2021. Bacteriocins produced by LAB isolated from cheeses within the period 2009–2021: a review. Probiotics Antimicrob. https://doi.org/10.1007/s12602-021-09825-0.

Tymoszewska, A., Ovchinnikov, K.V., Diep, D.B., Słodownik, M., Maron, E., Martínez, B., Aleksandrzak-Piekarczyk, T., 2021. *Lactococcus lactis* resistance to aureocin A53- and enterocin L50-like bacteriocins and membrane-targeting peptide antibiotics relies on the YsaCB-KinG-LlrG four-component system. Antimicrob. Agents Chemother. 65, e00921-21. https://doi.org/10.1128/AAC.00921-21.

Vasilchenko, A.S., Valyshev, A.V., 2019. Pore-forming bacteriocins: structural-functional relationships. Arch. Microbiol. 201, 147–154. https://doi.org/10.1007/s00203-018-1610-3.

Wang, C., Yang, J., Zhu, X., Lu, Y., Xue, Y., Lu, Z., 2017. Effects of *Salmonella* bacteriophage, nisin and potassium sorbate and their combination on safety and shelf life of fresh chilled pork. Food Control 73, 869–877. https://doi.org/10.1016/j.foodcont.2016.09.034.

Wiedemann, I., Breukink, E., van Kraaij, C., Kuipers, O.P., Bierbaum, G., de Kruijff, B., Sahl, H.G., 2001. Specific binding of nisin to the peptidoglycan precursor lipid II combines pore formation and inhibition of cell wall biosynthesis for potent antibiotic activity. J. Biol. Chem. 276, 1772–1779. https://doi.org/10.1074/jbc.M006770200.

Younes, M., Aggett, P., Aguilar, F., Crebelli, R., Dusemund, B., Filipič, M., Frutos, M.J., Galtier, P., Gundert-Remy, U., Kuhnle, G.G., Lambré, C., Leblanc, J., Lillegaard, I.T., Moldeus, P., Mortensen, A., Oskarsson, A., Stankovic, I., Waalkens-Berendsen, I., Woutersen, R.A., Wright, M., Herman, L., Tobback, P., Pizzo, F., Smeraldi, C., Tard, A., Papaioannou, A., Gott, D., 2017. Safety of nisin (E 234) as a food additive in

the light of new toxicological data and the proposed extension of use. EFSA J. 15, 5063. https://doi.org/10.2903/J.EFSA.2017.5063.

Yüksel, F.N., Buzrul, S., Akçelik, M., Akçelik, N., 2018. Inhibition and eradication of *Salmonella* Typhimurium biofilm using P22 bacteriophage, EDTA and nisin. Biofouling 34, 1046–1054. https://doi.org/10.1080/08927014.2018.1538412.

Zampara, A., Sørensen, M.C.H., Grimon, D., Antenucci, F., Vitt, A.R., Bortolaia, V., Briers, Y., Brøndsted, L., 2020. Exploiting phage receptor binding proteins to enable endolysins to kill Gram-negative bacteria. Sci. Rep. 10, 12087. https://doi.org/10.1038/S41598-020-68983-3.

Zheng, J., Ganzle, M.G., Lin, X.B., Ruan, L., Sun, M., 2015. Diversity and dynamics of bacteriocins from human microbiome. Env. Microbiol. 17, 2133–2143. https://doi.org/10.1111/1462-2920.12662.